Concurrent training in endurance athletes: the acute effects on muscle recovery capacity, physiological, hormonal and gene expression responses post-exercise

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Concurrent Training in Endurance Athletes: The Acute Effects on Muscle Recovery Capacity, Physiological, Hormonal and Gene Expression Responses Post-Exercise

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Declaration

I hereby declare that the work presented in this thesis is the original work of the author except as acknowledged in the text and has not been submitted, either in whole or in part, for a degree at this or any other university.

_____________________
Glen Bede Deakin
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Abstract

The research presented in this thesis examined the issue of the compatibility of strength and endurance training within one training regime, termed concurrent training, in recreational cyclists. Various research designs used in the previous literature resulted in inconclusive findings. The overall aim of this thesis was therefore to examine, in three systematically designed studies, the effects of various components of concurrent training regimes on cycling efficiency and recovery, and to identify some of the mechanisms that may be responsible for the interference or impedance of strength and/or endurance adaptations.

STUDY 1

The acute effects of strength training on the recovery of muscle force generating capacity and cycling efficiency, post-training

Previous studies on the acute effects of prior endurance exercise on muscle force-generating capacity have shown a reduction in muscle force generating capacity both immediately as well as in the hours post-exercise. The aim of this study was to examine the acute residual physiological effects of strength training on cycling efficiency and muscle force generating capacity three hours, and on blood variables three and 24 hours, post-training. This study consisted of two components: Experiment 1A examined varying intensities (high and low) and modes (weight lifting and hill cycling) of strength training, whilst Experiment 1B examined different durations (30 and 60 minutes) of strength training. In Experiment 1A nine male cyclists (age 23.6 ± 5.1 years) who were doing concurrent strength and endurance training completed a control trial of a discontinuous incremental cycle efficiency test (CE) one-week prior to the completion of three treatment trials over a three-week period. The three treatment trial days consisted of single sessions of 1) lower body strength (S), leg-press 6 sets x 6 repetition maximum (RM), 2) lower body strength endurance (SE), leg-press 6 sets x 20 repetitions (equal work as per S session) and 3) hill cycle (H) training, 6 sets x 20 seconds of cycling at a workload corresponding to twice the work of the S protocol. Three hours following each of the strength training sessions, the subjects completed a cycling efficiency
test, at the same time as that in the control day trial during which expired respiratory gases, heart rate (HR), blood lactate (BL) concentration and tympanic temperature (TT), as well as gross (GE) and net (NE) cycle efficiency were measured. Prior to the strength training sessions and the efficiency test, the subjects completed three maximal voluntary knee-extension contraction (MVC) trials in conjunction with three voluntary and involuntary muscle activation (MA) trials using a twitch interpolation technique. Blood specimens were collected prior to and 24 hours post the strength training sessions for the determination of plasma creatine kinase (CK) activity. In Experiment 1B, the week following the completion of Experiment 1A, seven of the subjects completed a fourth strength training protocol (SUL), consisting of upper and lower body exercises: leg-press 6 sets x 6 RM, bench-press 4 sets x 6 RM, and lat pull-down 4 sets x 6 RM.

The results of Experiment 1A showed a non-significant trend of higher post-training CK levels after the S protocol compared to the SE and H protocols (p=.194). A significant reduction in MVC mean torque was found three hours post the S protocol (p<.05) but not the SE or H protocols. A reduction in the superimposed and control twitches paralleled those of the MVC torque for all protocols, indicating an element of peripheral fatigue. A greater physiological cost was found during the efficiency test following the S protocol for BL concentration, respiratory responses, and TT, as well as greater reductions in gross-body-mass (GBM), GE and NE compared to the efficiency test after the other training protocols. However, HR showed a significantly higher response (p<.05). The results of Experiment 1B showed significant (p<.05) increases in CK for the S and SUL protocols but not the SE and H protocols. No significant difference was found between the four training protocols for mean MVC torque or MA. However, the SUL, S and H protocols all recorded significant (p<.05) changes in mean torque when the respective reductions are expressed as a percentage of the pre-values, compared to the SE protocol. The SUL protocol showed a consistently higher physiological cost of cycling compared to the other protocols. It was concluded that the high-intensity S protocol had a greater residual effect on muscle force generating capacity and the physiological cost of cycling than the low-intensity SE protocol when equated for work volume. Further, that the 60-minute high-intensity SUL protocol had a greater residual physiological effect than the 30-minute high-intensity S protocol of similar intensity but a similar effect on the recovery of force generating capacity. It was also concluded that conventional high-intensity strength training had a greater physiological effect on cycling exercise than hill cycling strength training.
STUDY 2

The acute effect of the sequence of strength and endurance training on muscle force generating capacity and cycling performance, post-training

The aim of this study was to examine the acute residual physiological effects of two sequences of strength and endurance training completed on the same day on muscle force generating capacity and cycling efficiency three hours post-training and blood variables three and 24 hours post-training. Eight male cyclists (age 23.9 ± 5.6 years) who were doing concurrent strength and endurance training completed a control day with no training sessions, followed by two sequence days, weight/cycle (WC) and cycle/weights (CW), in random order over a three-week period. The 60-minute weight (W) training session was identical to that used in Experiment 1B of Study 1 for the SUL protocol, whilst the cycle (C) session consisted of 60-min of loaded cycling at 60% of VO2 max on a cycle ergometer. The training sessions were separated by a period of three hours. Three hours following the second training session the subjects completed a cycling efficiency test at the same time as that in the control day trial. The same physiological variables were measured during the C training session and the efficiency tests as outlined for Study 1. Prior to each training session as well as the efficiency test, the subjects completed three MVC knee-extension and MA trials as described for Study 1. Blood specimens were collected prior to, immediately following and 24 hours post each training session for the determination of resting and post-training BL and plasma CK activity. Serum testosterone and cortisol concentrations were also determined from pre-training and efficiency test blood specimens.

The results indicated a higher pre (p<.05) and post BL concentration for the W session of the CW sequence compared to the WC sequence, respectively. Respiratory rate, HR, BL, minute-ventilation, VO2, TT and NE were all higher whilst GE was notably lower during the C session of the WC sequence compared with the CW sequence. No significant difference was found between the two sequence days for CK. The W session of the CW sequence produced a similar reduction in peak and mean knee-extension torque to that of the WC, respectively. In contrast, the C session of the WC sequence showed a greater reduction in peak and mean torque than that found for the CW sequence, which showed no change in peak and < 1.5%
reduction in mean torque, resulting in a significantly greater reduction (p<.05) of -8.28% from pre-training to three-hour post-training for the WC sequence compared to the CW sequence. No significant difference was found between the three trial days for MA. However, a reduction in the superimposed and control twitches paralleled those of the MVC torque for both the WC and CW sequences. The WC showed greater changes than the CW across the majority of the variables measured during the efficiency test. A significantly lower (p<.05) post-training testosterone concentration was found for the CW compared to the WC sequence. A significant reduction (p<.05) in cortisol was found for the WC and CW sequence days compared to the control day and a lower post-training testosterone/cortisol (T/C) ratio was found for the CW compared to the WC sequence. It was concluded that there was an increased physiological stress during those sessions completed second in the training sequence compared to when they were completed first, irrespective of the type of training. Further, it was concluded that the recovery of force generating capacity and physiological parameters following the training sessions are dependent on the sequence of training, with the sequence WC requiring a greater recovery period than the CW.

STUDY 3

The effect of the sequence of strength and endurance training on hormonal and gene expression responses and muscle glycogen content post-training

There have been limited investigations of the mechanisms for the possible impedance of strength and endurance development. The aim of this study was to determine whether the sequence of completing strength and endurance training sessions on the same day affected hormonal responses and skeletal muscle gene expression as well as muscle glycogen content post-training. Eight male weight-trained cyclists (age 26.1 ± 2.4 years) completed four trial days over a five-week period: single weight (W) and endurance cycle (C) training and two sequence days, WC and CW. The 60-minute W training session consisted of three exercises: leg-press 6 sets x 6 RM, leg-curl 4 sets x 6 RM, and leg-extension 4 sets x 6 RM. The C training consisted of 170 minutes of cycling at 60% of VO2 max. The sequence day training sessions were separated by a period of three hours. Blood and muscle specimens were collected before and after each training session for determination of BL and plasma testosterone and cortisol concentrations as well as muscle glycogen content and expression
levels of selected genes associated with muscle growth and metabolic function (no muscle specimens were collected on control days). During the W sessions, HR was measured whilst the same physiological variables were measured during the C training session as for Study 2.

Higher HR and BL responses were found in those sessions completed second in the training sequences. Higher responses were also found for all variables during the C session for the WC sequence compared to the CW sequence. Furthermore, the respiratory responses of WC progressively increased at a faster rate over the last 60-90 minutes of the 170-minute C session, compared to the relatively stable responses for the CW sequence. A notable reduction in GE and NE for the WC sequence was also found along with a reduction in RER during the C session. A significant difference (p<.05) was found between the pre and post W time points, with the CW sequence showing considerably lower glycogen levels than the WC sequence, respectively due to a significant reduction produced by the prior C session of ~70% from pre- to post-training. No significant difference was found between the remaining time points. Both sequence days showed temporal increases in testosterone and cortisol following the second training sessions compared to the control days. Even though no significant difference was found between the two training sequences for testosterone or cortisol, the WC sequence showed a greater response post the second training session than the CW sequence. The WC sequence also produced a reduced T/C ratio compared to the CW sequence in the hours following the second training session. The muscle regulatory factor genes, MyoD and myogenin were affected by the sequence of training, responding more to the CW sequence than the WC sequence, especially at the 25 hour post W time point where significant ~5-6 fold increases in mRNA content were found. In contrast, the genes PDK4, HKII, PGC1 and LPL, which are associated with metabolic functions, responded more to the WC sequence than the CW sequence. The remaining genes associated with muscle metabolic function did not respond more to one training sequence than another. It was concluded that the sequence of training affected the expression of some skeletal muscle genes associated with growth and metabolic functions, but did not directly affect the level of muscle glycogen depletion post the training sessions. However, indirectly affected the choice of substrate during the C session of the WC sequence. It was also concluded that the completion of the second bout of training, irrespective of whether it was strength or endurance orientated, altered the testosterone and cortisol responses compared to the single mode training sessions. Further, that the hormonal response was greater for the WC sequence than the CW sequence.
List of Publications

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<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATPS</td>
<td>ambient temperature and pressure, saturated with water vapour</td>
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<tr>
<td>BL</td>
<td>blood lactate concentration</td>
</tr>
<tr>
<td>BTPS</td>
<td>body temperature and pressure, saturated with water vapour</td>
</tr>
<tr>
<td>C</td>
<td>endurance cycle training</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<tr>
<td>CE</td>
<td>control efficiency</td>
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<tr>
<td>CHO</td>
<td>carbohydrate</td>
</tr>
<tr>
<td>CK</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>COMB</td>
<td>combined strength and endurance training</td>
</tr>
<tr>
<td>CP</td>
<td>creatine phosphate</td>
</tr>
<tr>
<td>CW</td>
<td>cycle then weight training</td>
</tr>
<tr>
<td>DEPC</td>
<td>diethyl pyrocarbonate</td>
</tr>
<tr>
<td>E</td>
<td>endurance training</td>
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<tr>
<td>ECG</td>
<td>electrocardiograph</td>
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<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
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<tr>
<td>e.g.</td>
<td>example</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyography</td>
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<tr>
<td>EPOC</td>
<td>excess post-exercise oxygen consumption</td>
</tr>
<tr>
<td>ES</td>
<td>electrical stimulation</td>
</tr>
<tr>
<td>GBM</td>
<td>gross body mass</td>
</tr>
<tr>
<td>GE</td>
<td>gross efficiency</td>
</tr>
<tr>
<td>GT</td>
<td>guanidinium thiocyanate</td>
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<tr>
<td>H</td>
<td>hill cycle training</td>
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<tr>
<td>HR</td>
<td>hear rate</td>
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<tr>
<td>ICC</td>
<td>intraclass correlation coefficient</td>
</tr>
<tr>
<td>i.e.</td>
<td>that is</td>
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<tr>
<td>iEMG</td>
<td>integrated electromyography</td>
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<tr>
<td>MA</td>
<td>muscle activation</td>
</tr>
<tr>
<td>MHC</td>
<td>myosin heavy chain</td>
</tr>
<tr>
<td>MPT</td>
<td>maximal peak torque</td>
</tr>
<tr>
<td>MRFs</td>
<td>myogenic regulatory factors</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>MVC</td>
<td>maximal voluntary contraction</td>
</tr>
<tr>
<td>n</td>
<td>number of subjects</td>
</tr>
<tr>
<td>NC</td>
<td>no change</td>
</tr>
<tr>
<td>NE</td>
<td>net efficiency</td>
</tr>
<tr>
<td>%</td>
<td>percent</td>
</tr>
<tr>
<td>r</td>
<td>Pearson’s Product Moment correlation coefficient</td>
</tr>
<tr>
<td>RER</td>
<td>respiratory exchange ratio</td>
</tr>
<tr>
<td>RM</td>
<td>repetition maximum</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>ROM</td>
<td>range of motion</td>
</tr>
<tr>
<td>RPE</td>
<td>rating of perceived exertion</td>
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RR: respiratory rate
S: strength training
SE: strength endurance training
SD: standard deviation
STPD: standard temperature and pressure, dry
SUL: upper and lower body strength training
TEM: technical error of measurement
TT: tympanic temperature
TW: twitch interpolation
U/COMB: combined upper body strength and endurance training
VCO2: volume of carbon dioxide production per minute
VE: minute ventilation
VO2: volume of oxygen consumption per minute
VO2 max: maximal oxygen uptake
WC: weight then cycle training
W: weight training
WL: workload

**Gene Abbreviations**

CPT1: carnitine palmitoyltransferase
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
GYS: glycogen synthase
HKII: hexokinase II
IGF1& 2: insulin-like growth factor 1& 2
LDH-H: lactate dehydrogenase-H
LDH-M: lactate dehydrogenase-M
LPL: lipoprotein lipase
MHCI: myosin heavy chain I
MHCIIa: myosin heavy chain IIA
MHCIIx: myosin heavy chain IIX
PDK4: pyruvate dehydrogenase kinase 4
PGC1: proliferator-activated receptor gamma coactivator-1
UCP3: uncoupling protein 3

**Units of Measurement**

b.min⁻¹: beats per minute
br.min⁻¹: breaths per minute
g: gram
kcal: kilocalorie
kg: kilogram
km: kilometre
L.min⁻¹: litres per minute
m: metre
i L: microlitre
min: minute
mL: millilitre
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>mL·h(^{-1})</td>
<td>millilitres per hour</td>
</tr>
<tr>
<td>mL·kg(^{-1})·min(^{-1})</td>
<td>millilitres per kilogram per minute</td>
</tr>
<tr>
<td>mL·min(^{-1})</td>
<td>millilitres per minute</td>
</tr>
<tr>
<td>mmHg</td>
<td>millimetre of mercury</td>
</tr>
<tr>
<td>mmol·L(^{-1})</td>
<td>millimole per litre</td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>ng·mL(^{-1})</td>
<td>nanogram per millilitre</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>°C</td>
<td>temperature in degrees Celsius</td>
</tr>
<tr>
<td>U·L(^{-1})</td>
<td>Units per litre</td>
</tr>
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CHAPTER 1

Introduction

1.1. Background

The primary objective of sports training is to stress various bodily systems to bring about positive adaptation in order to enhance sporting performance (Friel, 1998; Rushall & Pyke, 1990). To achieve this objective, coaches and athletes systematically apply a number of training principles including overload, specificity and progression (Baechle, Earle, & Wathen, 2000; Rushall & Pyke, 1990; Wilson, 1994b), organised through what is commonly termed periodisation (Bompa, 1994). The application of these principles involves the manipulation of various programme design variables including choice of exercise, order of training activities/exercises, training intensity (load and repetition), rest periods between sets and activities/exercises and training frequency and volume in order to provide periods of stimulus and recovery, with the successful balance of these factors resulting in positive adaptation (Baechle et al., 2000; Fleck & Kraemer, 1987; Kraemer, Deschenes, & Fleck, 1988; Wilson, 1994b).

Whilst the abovementioned training principles are employed for both endurance and strength training regimes, the physiological adaptations for both are notably different due to differences in the application of programme design variables (Dudley & Fleck, 1987). Endurance training programmes such as those used for running or cycling typically involve the performance of high-repetition, low-resistance exercise continuously over long periods of time (e.g. 1-2 hours) (Dudley & Fleck, 1987). The intention of this type of training is to increase aerobic capacity (maximal oxygen uptake (VO₂ max), efficiency and economy) through physiological changes including increased muscle capillary and mitochondrial density and enzyme activity in the respiratory pathway (Holloszy & Coyle, 1984; Kraemer, 2000a; Terjung, 1995). Due to the low level of resistance utilised, endurance training produces very little change in a muscle’s strength capabilities (Fitts & Widrick, 1996). In contrast, strength training typically involves the performance of high-resistance, low-repetition exercises to
produce increases in muscle strength, hypertrophy and motor performance (Dudley & Fleck, 1987; Fleck & Kraemer, 1997). Associated with these changes are increases in muscle fibre size (Hather, Tesch, Buchanan, & Dudley, 1991; MacDougall, Sale, Alway, & Sutton, 1984), a reduction in mitochondrial density (MacDougall, Sale, Moroz, Elder, & Sutton, 1979; Tesch, 1987), an alteration of the ratio of type II fibres (Adams, Hather, Baldwin, & Dudley, 1993; Fleck & Kraemer, 1988; Staron et al., 1994; Staron et al., 1991) and little or no change in aerobic capacity (Fleck & Kraemer, 1987).

In light of these differences in adaptations to endurance and strength training, the question needs to be asked, are athletes who supplement their sport specific training with other forms of physical conditioning in order to enhance their sporting performance (e.g. endurance athletes engage in strength training and vice versa), optimising their potential for adaptation to both forms of conditioning? Whilst an array of books have been published suggesting that strength training can increase strength levels and enhance sporting performance, little is known about the compatibility of strength and endurance training within one training regime, commonly termed concurrent training (Hickson, 1980). Further, it is not clear whether concurrent training sessions compromise, enhance or have no influence on physiological responses and subsequent adaptations of the respective training sessions compared to single mode strength and endurance training regimes (Dudley & Fleck, 1987). If concurrent training regimes do enhance or compromise strength and/or endurance adaptation, the question is then, does concurrent training require a different set of programme design variables compared to single mode strength and endurance training regimes? For example, is the order that the strength and endurance training sessions are completed important?

Since Hickson (1980) first investigated the impact of concurrent training on strength and endurance development in untrained subjects and reported compromised strength gains compared to a strength trained only group, a number of studies have investigated whether the combination of strength and endurance training sessions are compatible (Bell, Petersen, Wessel, Bagnall, & Quinney, 1991b; Craig, Lucas, Pohlman, & Stelling, 1991; Dolezal & Potteiger, 1998; Dudley & Djamil, 1985; Kraemer et al., 1995; Sale, Jacobs, MacDougall, & Garner, 1990b; Sale, MacDougall, Jacobs, & Garner, 1990a). To date the findings are inconclusive, however, the current evidence suggests that there is interference or impedance between strength and endurance adaptation, in particular strength, when the two forms of
training are included in the same training regime (Leveritt, Abernethy, Barry, & Logan, 1999).

The inability to determine the compatibility of the two types of training from the current literature can be attributed to the large variety of research designs used in previous investigations of concurrent training. To date the designs have incorporated a wide selection of independent variables including training modalities (strength – isoinertial, isokinetic; endurance – running, cycling and arm cranking), training protocols (upper/lower body exercises, training volume/intensities, types of equipment used - hydraulic, free weights, weight machines), training sequences (strength/endurance, endurance/strength) and training schedules (same/separate days) (Leveritt et al., 1999). Furthermore, various training periods (7 to 22 weeks) and subject populations (trained/untrained) have been utilised. A change in anyone of these variables may alter the level of stress that a subject experiences, the acute response to the training stimulus imposed and hence the level of strength and/or endurance adaptation. Thus providing a source for varying results when comparing the effects of concurrent training.

Because prior investigations have not systematically examined alterations in independent variables, for example the intensity of training or the sequence and scheduling of concurrent training sessions, it remains unclear as to the extent that variations in the independent variables impact upon the level of strength and endurance response and adaptation. Collins and Snow (1993), in one of the few studies that manipulated the sequence of training by comparing endurance/strength to strength/endurance within the same session over a 7 week period, reported that there was no significant difference between the two sequences as assessed by VO2 max, one-repetition maximum (1 RM) bench press, arm-curl and leg-press strength. Whilst this suggests that the development of strength and endurance may be independent of the sequence of training, further research is required of the various combinations of training sequences and schedules as well as the other independent training variables, to determine the extent to which training protocols, sequences or schedules influence concurrent training responses and adaptations.

In addition, the majority of prior concurrent training studies have focused on the completion of weeks of training and the subsequent level of adaptation over the course of a combined strength and endurance training regime. The acute strength or endurance responses following
a concurrent training session have not been examined. To date most studies that have examined the acute responses to multiple strength training sessions on the same day (Häkkinen, 1992) or following some form of endurance type exercise (Bentley, Zhou, & Davie, 1998; Sahlin & Seger, 1995) have done so from the perspective of maximal voluntary force generating capacity. To date there have been no investigations that have examined the acute physiological effects of prior strength training on endurance performance. However, evidence from previous studies that have completed two strength (Häkkinen, 1992) or endurance training sessions (Ronsen, Haug, Pedersen, & Bahr, 2001) in the one day or other methods of physical training other than resistance training over separate days (Gleeson, Blannin, Zhu, Brooks, & Cave, 1995), suggest that subsequent activities undertaken using the same muscle groups as those used in a prior activity, maybe adversely affected because of residual fatigue (Häkkinen, 1992) or a higher relative exercise stress (Gleeson et al., 1995; Ronsen et al., 2001).

A number of training modalities and protocols have been used in concurrent training studies (Craig et al., 1991; Dudley & Djamil, 1985; Hortobágyi, Katch, & Lachance, 1991; Sale et al., 1990b). However, there have been no investigations that have examined the impact of using a within-sport strength training mode during a concurrent training program. Whilst the effects of concurrent training, in the form of “in-water” strength and endurance training on swimming endurance performance are unknown (Tanaka & Swensen, 1998), data exists that indicates that swim strength training (in-water) can improve a swimmer’s velocity over distances up to 200 metres (Toussaint & Vervoorn, 1990) as well as stroke force and distance per stroke (Tanaka, Costill, Thomas, Fink, & Widrick, 1993; Toussaint & Vervoorn, 1990). This data suggests that the modality of strength training may play a greater role in improving the compatibility of strength and endurance training than using traditional strength training methods. However, further research is required for swimming and other types of endurance sports such as cycling and running before the extent of compatibility of within-sport strength and endurance training can be known.

The inconsistency of research findings due to the use of a wide range of training variables, project design and subject populations has hampered the identification of the mechanisms that may be responsible for interfering or inhibiting the development of strength and/or endurance when trained concurrently compared to single mode strength and endurance training. However, investigators have proposed a number of mechanisms thought responsible for the
current findings, both chronic and acute, based on the differences in physiological responses to strength and endurance training. The proposed chronic mechanisms include alterations in the concentrations of various anabolic/catabolic hormones (testosterone/cortisol), differences in the organisation of neuromuscular recruitment patterns and changes in muscle fibre hypertrophy and transformation processes (Chromiak & Mulvaney, 1990). The acute mechanism thought responsible is residual fatigue incorporating such sources as neuromuscular fatigue, muscle damage and glycogen depletion (Chromiak & Mulvaney, 1990; Leveritt et al., 1999).

The foundation for these proposed mechanisms has come predominantly from the extrapolation of responses and adaptations found with single mode strength and endurance training studies, as there have been limited concurrent training studies conducted that have measured metabolic and/or morphological responses and adaptations to concurrent strength and endurance training (Kraemer et al., 1995; Nelson, Arnall, Loy, Silvester, & Conlee, 1990; Sale et al., 1990b). Further, those that have attempted to investigate the mechanisms behind the adaptations, have only examined a few chronic mechanisms, namely changes in muscle fibre hypertrophy and transformation at the gross level (Kraemer et al., 1995; Nelson et al., 1990; Sale et al., 1990a) and to a lesser extent hormonal alterations (Bell, Syrotuik, Martin, Burnham, & Quinney, 2000; Kraemer et al., 1995). This has added to the difficulty in pinpointing the mechanism or mechanisms responsible for the possible inhibition of strength and endurance development because the monitoring of the resultant level of adaptation to a stimulus does not necessarily provide an indication as to the source of the change. Subsequently, possible sources such as changes in the level of protein synthesis following altered gene expression activity of selected muscle growth and metabolic factors following concurrent training have not been examined.

Whilst it is unlikely that any individual mechanism is solely responsible for interfering in strength and/or endurance development during concurrent training, the role that each proposed mechanism plays in this complex interaction of training, response and adaptation is unknown.
1.2. Statement of the Problem

Investigations into the effects of concurrent training on strength and endurance development have been limited to training studies examining the level of adaptation over time or the acute effects of prior endurance exercise on subsequent muscle force generating capacity. To date there has been no investigation that has investigated the acute physiological effects of prior strength training on endurance performance. Further, because there has been no systematic examination of selected combinations of independent variables, the influence of different intensities, types and sequences of strength and endurance training on strength and endurance responses and development are unclear.

The knowledge gained to date on the possible mechanisms underlying the interference or impedance of strength and endurance development is based on the extrapolation of single mode strength and endurance training studies or limited concurrent training studies. Subsequently, the role that such mechanisms as residual fatigue from muscle damage and glycogen depletion and changes in hormone concentrations (testosterone and cortisol), in addition to possible alterations in protein synthesis due to changes in skeletal muscle gene expression, have on concurrent training responses and adaptations are still unclear.

Consequently, the aims of this investigation were:

- To systematically examine and compare the acute residual physiological effects of,
  
  i. varying intensities of strength training (high and low);
  
  ii. different durations of strength training (30 and 60 minutes);
  
  iii. different modes of strength training (weight lifting and cycling); and,
  
  iv. sequences of strength and endurance training completed on the same day,
on muscle force generating capacity and cycling efficiency three hours post-training and blood variables three and 24 hours post-training.

- To determine whether the sequence of completing strength and endurance training sessions on the same day would alter,

  i. the hormonal responses of testosterone and cortisol compared to single mode training sessions post-training;

  ii. the level of skeletal muscle glycogen depletion and recovery, during and following the training sessions, respectively; and

  iii. the level of expression of selected genes associated with skeletal muscle growth and metabolism, post-training.

1.3. Hypotheses

The hypotheses of this investigation were:

- The intensity, duration and mode of strength training would influence the acute residual physiological effects on muscle force generating capacity and cycling efficiency three hours post-training and blood variables three and 24 hours post-training. Specifically that,

  i. high-intensity strength training sessions would have a greater residual physiological effect than low-intensity strength training sessions when equated for work volume;

  ii. longer duration high-intensity strength training sessions would have greater residual physiological effect than lesser duration high-intensity strength training sessions; and/or
iii. traditional strength training incorporating both eccentric and concentric contractions would have a greater physiological effect than hill cycling strength training, which incorporates concentric contractions only.

- The sequence of completing strength and endurance training sessions would affect,

i. the physiological stress of the second training session compared to when the training sessions are completed first;

ii. the recovery dynamics of both force generating capacity and physiological parameters after the second training session;

iii. the hormonal responses post-training of testosterone and cortisol compared to single mode training sessions;

iv. the level of skeletal muscle glycogen depletion and recovery, during and following the training sessions, respectively; and/or

v. the level of expression of selected genes associated with skeletal muscle growth and metabolism, post-training.

1.4. Significance of the Study

This investigation systematically examined the effects of different types and modes of strength training on cycling performance as well as the sequence and scheduling of training sessions and will contribute to our knowledge of how the choice of training variables in a concurrent training regime may influence strength and endurance responses and the time course of these responses post-training.

Further, this study provided insight into the impact that changes in the abovementioned training variables had on skeletal muscle glycogen content, hormonal responses and expression of genes important in muscle growth and metabolism and as such provided a better understanding of how these mechanisms would affect strength and/or endurance development.
The knowledge gained from this study would benefit coaches and athletes, as it would assist in devising more optimal methods of programme design to enable the maximisation of both strength and endurance adaptation potential.

1.5. Research Design

Three studies were designed to examine specific aspects of concurrent training for weight trained recreational cyclists. The aim and design of each study was as follows:

- **Study 1** - examined the effects of varying intensities, durations and modes of strength training on subsequent cycling performance assessed via changes in cycling efficiency, muscle recovery capacity and blood variables post-training. This study consisted of two experiments:
  
  - **Experiment 1A** – nine subjects completed one of three short duration (~30 minutes) lower body strength training protocols followed three hours post-training by a discontinuous incremental cycling efficiency test.
  
  - **Experiment 1B** – seven of the subjects from Experiment 1A completed a fourth training protocol to examine the impact of a longer duration (60-minute) high-intensity training protocol on the same variables as those of the three shorter duration (30-minute) training protocols.

- **Study 2** - was an extension of Study 1 and was designed to examine the impact that the sequence of strength and endurance training sessions, separated by periods of rest (three hours), had on the same assessment criteria as used in Study 1 except for the inclusion of selected hormonal responses. Eight subjects completed two different training sequences (strength/endurance and endurance/strength) over a two-week period, incorporating weight and cycling endurance training sessions of one-hour in duration. Three hours after the completion of the training session the subjects completed a second training
session. This was followed three hours later by a cycling efficiency test, as used in Study 1.

- **Study 3** - Using a similar design to that of Study 2, except that there was no cycling efficiency test during the training days, Study 3 examined three of the possible mechanisms (hormone responses, muscle glycogen depletion and alterations in mRNA of selected genes in skeletal muscle) that might contribute to the interference of strength and endurance responses and adaptations found in concurrent training studies.

Studies 1 and 2 were carried out at Southern Cross University, Lismore, Australia, and Study 3 was conducted at the Copenhagen Muscle Research Centre, Denmark.

**1.6. Limitations**

1. Due to resource constraints, subject and laboratory availability, the number of tests to be performed and the length of the testing periods, sample sizes were limited to 10 or less for all three studies.

2. All the subjects were volunteers and therefore, were not a random sample of cyclists who concurrently engaged in strength training.

3. The use of laboratory cycle ergometers, while being accurate and standardised for each subject, they do not replicate exactly the normal cycling set up of the cyclists' personal riding position and thus may have hampered the subjects' performance due to physical and/or psychological disturbances.

4. Due to the requirement of multiple sessions over a period of weeks and at different times of the day, performance may be influenced by biological rhythm. However, every effort was made to limit the impact of biological rhythm fluctuations on the results by testing subjects on the same day of the week, at the same time of day for each session and controlling for exercise and food intake.
5. Recovery from exercise as well as variations in hormonal concentrations is affected by factors such as diet, daily activity and stress. Whilst every effort was made to control for these influences by controlling exercise activity and food and fluid intake on testing days, it is possible that some uncontrolled outside influence such as family or emotional issues may have impacted upon the results.

1.7. Delimitations

1. The subject sample represented a small section of the amateur weight trained endurance cyclist population. That is, males aged between 18 to 40 with weight training and cycling experience and currently cycling two to more times per week. Therefore, whilst the results may reflect the above sample population, the extrapolation of the results directly to groups outside the sample population may not always be valid.

2. The testing times after the strength and/or endurance training sessions in each of the studies was pre-determined from the viewpoint of resource constraints. The timing of such testing may not have been as beneficial in detecting physiological changes as a more frequent or longer testing protocol. Therefore, the extrapolation of the results outside these time frames may be somewhat limited.
CHAPTER 2

Review of the Literature

The Review of the Literature is divided into three sections. The first section introduces the physiological responses and adaptations to individual strength and endurance training sessions. The second section outlines the findings of concurrent training research including the effects of prior bouts of endurance or strength training on subsequent muscle force generating capacity and recovery dynamics. Finally, the third section presents possible mechanisms for compromised responses and adaptations with concurrent training. Furthermore, due to the vast volume of literature in relation to strength and endurance training responses and adaptations, the Review of the Literature could only cover those areas that are deemed most relevant to the concept of concurrent training.

2.1. Introduction

An effective training programme requires a combination of the intensity, duration, frequency and mode of exercise to overload a body system and cause adaptation (McCafferty & Horvath, 1977; Tanaka & Swensen, 1998). The training induced adaptations that occur within an individual are specific to the training programme employed, known as the specificity principle of training (Hawley & Burke, 1998; McCafferty & Horvath, 1977). The chronic physiological change that occurs as a body adapts to a stimulus that is imposed repeatedly, is termed an adaptation to training (Bompa, 1994; Fleck & Kraemer, 1997). Whilst the acute physiological change that occurs in response to a single bout of a stimulus is termed a response to training (Fleck & Kraemer, 1997).

Regardless of whether the intention of training is for strength or endurance adaptation, a common principle of training is that, the accumulation of the acute responses to a stimulus is what will determine the resultant level of adaptation (Brooks, Fahey, White, & Baldwin, 2000; Fleck & Kraemer, 1997). The response process has been illustrated as an overcompensation cycle (Figure 2.1), whereby the application of a stimulus results in an individual experiencing fatigue (Phase 1) and then compensating fully (Phase 2), followed by
an overcompensation phase (Phase 3) and finally an optimal time during the overcompensation phase for a repeated stimulus (Bompa, 1994). If another stimulus is not applied in the optimal time period, then involution occurs (Phase 4) and with it a loss of the benefits obtained during the overcompensation phase (Bompa, 1994), the result of which maybe a lower rate of improvement in an individual’s adaptation to a stimulus.

Figure 2.1. The overcompensation cycle following a training stimulus {Adapted from Bompa (1994), modified from original source Yakovelv (1967)}.

In contrast, the application of repeated bouts of stimuli prior to an individual recovering adequately that is, reaching the peak of the overcompensation phase, may result in interruption of the stimulus response and hence a retardation of the level of overcompensation reached post-exercise (Figure 2.2).

If this process was to continue on successive applications of the training stimuli, then the level of adaptation would be reduced compared to if adequate recovery and appropriate timing of the application of the repeated stimulus was provided. That is, a slower rate of improvement in that particular physiological component (Bompa, 1994). Consequently, it is the acute response to a stimulus and the subsequent application of repeated stimuli that determines the level of adaptation over time and hence the performance outcome (Brooks et al., 2000; Fleck & Kraemer, 1997). In light of this, the ability of the body to recover from a training stimulus
is important and as such consideration has to be given to the type of training performed and
the time intervals between training sessions.

![Diagram of Retardation of the level of overcompensation following the application of a second stimulus prior to the potential level of overcompensation from the first stimulus being reached.](image)

**Figure 2.2.** Retardation of the level of overcompensation following the application of a second stimulus prior to the potential level of overcompensation from the first stimulus being reached.

### 2.2. Physiological Recovery Responses to Endurance and Strength Exercise

It is well documented that following exercise, bodily processes do not immediately return to pre-exercise levels resulting in what is termed excess post-exercise oxygen consumption or more commonly referred to as EPOC (Åstrand & Rodahl, 1986; Brooks et al., 2000; Brooks, Hittelman, Faulkner, & Beyer, 1971; Gaesser & Brooks, 1984; McArdle, Katch, & Katch, 1996). The period of EPOC has been found to consist of two phases, with an initial period of rapid decline and then a prolonged period where the oxygen consumption slowly returns to resting levels (Krogh & Lindhard, 1920). The rapid EPOC component referring to where the factors contributing to EPOC are present for less than one hour whilst the prolonged period refers to where there is an increase in oxygen consumption for many hours post-exercise (Bahr, 1992; Børsheim, Knardahl, Høstmark, & Bahr, 1998). The literature indicates that the disturbance in homeostasis post-exercise during the period of rapid EPOC is the result of a number of factors (Brehm, 1988; Brooks et al., 2000; Brooks et al., 1971; Gaesser & Brooks, 1984; McArdle et al., 1996) including:
• Thermogenic effects of elevated body temperature
• Resynthesis of adenosine triphosphate (ATP) and creatine phosphate (CP) stores
• Resynthesis of glycogen from lactate
• Increased levels of circulation and ventilation
• Redistribution of ions within various body compartments
• Oxygen re-saturation of myoglobin and haemoglobin

Some of these factors, such as the re-saturation of myoglobin and haemoglobin with oxygen and the resynthesis of ATP and CP stores, are known to have a only a small influence on EPOC due to the replenishment of these factors taking only seconds to minutes to complete (Bahr, 1992). Other factors have been shown to take longer to return to the pre-exercise state, for example the resynthesis of glycogen from lactate. In a review of the metabolic bases of EPOC, Gaesser and Brooks (1984) outlined that, apart from the removal of lactate, the most influencing factor on EPOC was an elevation in body temperature because of its negative effect on phosphorylate-coupling efficiency, resulting in an increase in oxygen consumption to produce the same amount of ATP.

Other factors have been proposed to explain the prolonged period of EPOC in addition to increased levels of circulation and lung ventilation, which have been found to remain significantly elevated above resting levels for up to 12 hours post-exhaustive sub-maximal exercise (Bahr, Ingnes, Vaage, Sejersted, & Newsholme, 1987; Bahr & Sejersted, 1991). These other factors include the residual effects of elevated hormones (Bahr, 1992; Bangsbo & Hellsten, 1998; Mahlum, Grandmontagne, Newsholme, & Sejersted, 1986), a shift from carbohydrate to fat as a substrate source (Bahr, Hansson, & Sejersted, 1990; Wolfe, Klein, Carraro, & Weber, 1990), increased rates of substrate cycling (Bahr, 1992; Børsheim et al., 1998; Gaesser & Brooks, 1984) and the repair of damaged tissue (Børsheim et al., 1998; Schuenke, Mikat, & McBride, 2002). Whilst the effects of the repair of muscle damage on EPOC have not been extensively researched, it has been postulated that eccentric-based exercise, which has been shown to produce high levels of muscle damage (Armstrong, Ogilvie, & Schwane, 1983; Clarkson, Litchfield, Graves, Kirwan, & Byrnes, 1985; Hortobágyi & Denahan, 1989; Jamurtas et al., 2000; Newham, McPhail, Mills, & Edwards, 1983), may cause notable perturbations in homeostasis due to the increased energy
requirements associated with repair of the damaged tissue via increased protein breakdown and synthesis (Dolezal, Potteiger, Jacobsen, & Benedict, 2000).

Although there has been limited research conducted on the effects of elevated hormone levels on EPOC, increased levels of selected hormones such as cortisol, one hour after prolonged exercise have been reported, which may alter metabolic processes during the recovery period and thereby increase the level of oxygen consumption (Mæ hlum et al., 1986). Of the factors associated with the prolonged EPOC period, an increase in the rate of substrate cycling has been attributed as one of the most significant (Bahr, 1992), particularly with respect to the triglyceride-fatty acid metabolism, which has been shown to increase by as much as 300% (Bahr et al., 1990).

The current literature evidence indicates that EPOC varies according to the nature of the exercise that is, whether the exercise is strength- or endurance-orientated and the duration and intensity of the exercise (Børsheim et al., 1998; Brehm, 1988). Further, a comparison of leg versus whole body post-exercise oxygen consumption has shown that not only are the muscles directly involved in the exercise associated with EPOC but also other tissues not directly associated with the exercise (Bangsbo et al., 1990). Consequently, the effects of different intensities and durations of single bouts of endurance or strength training have on EPOC are outlined below to highlight the influence that the type of training has on the body’s recovery capacity post-exercise.

### 2.2.1. Endurance Exercise and EPOC

Since early investigations by Krogh and Lindhard (1913) of the time course of oxygen uptake following exercise, a number of studies have investigated the effects of exercise intensity (Brehm & Gutin, 1986; Gore & Withers, 1990; Sedlock, Fissinger, & Melby, 1989) and duration (Bahr et al., 1987; Gore & Withers, 1990; Mahlum et al., 1986; Quinn, Vroman, & Kertzer, 1994; Sedlock et al., 1989) on the EPOC response post-endurance (aerobic) orientated activities. In a study by Brehm and Gutin (1986) using trained subjects who walked or ran 3.2km at varying intensities (18-68% VO₂ max), it was found that EPOC was greater for running compared to walking and for high versus low intensities of exercise. Whilst there was a difference between the activities and intensities with respect to EPOC, all conditions returned to normal levels within an hour.
In a similar designed study to that of Brehm and Guitin (1986), Sedlock, Fissinger and Melby (1989) compared varying intensities and durations of cycling and reported comparable findings, that exercise intensity and duration influenced both the magnitude and duration of EPOC. They tested ten trained male triathletes during three cycling bouts at varying intensities consisting of high-intensity (75% of VO\textsubscript{2} max)/short-duration, low-intensity (50% of VO\textsubscript{2} max)/short-duration and low-intensity/long-duration. The duration of the short bouts was determined by the time taken to expand 300 kcal whilst the long-duration was the time taken to expend 600 kcal. Monitoring recovery VO\textsubscript{2} until it returned to baseline levels, it was found that when energy expenditure was held constant, the high-intensity/short-duration bout produced a greater EPOC and for a longer time period than the low-intensity/short-duration bout. As was the case with Brehm and Guitin (1986), post-exercise oxygen consumption returned to resting levels within an hour.

In contrast to the above studies, that reported that EPOC was elevated for less than one hour in duration following exercise, a study by Bahr et al (1987) investigated the impact of varying durations (20, 40 and 80 minutes) of cycling exercise at 70% of VO\textsubscript{2} max, EPOC was reported to have remained elevated for 12 hours. The investigators indicated that the magnitude of 12 hour EPOC was related to the duration of prolonged exercise and equalled approximately 14, 7 and 5% after 80, 40 and 20 minutes of exercise, respectively. This study confirmed an earlier report by Mæhlum et al (1986) who reported that EPOC was elevated above basal oxygen consumption by 14% for a period of 12 hours post 90 minutes of cycling at 70% of VO\textsubscript{2} max.

In a comprehensive study of exercise intensity and duration on post-exercise metabolism, Gore and Withers (1990) found that exercise intensity and duration were important factors of the magnitude of EPOC after treadmill exercise bouts of 20, 50 and 80 minute duration at intensities of 30, 50 and 70% of VO\textsubscript{2} max. They found that of the six 30 and 50% of VO\textsubscript{2} max trails using trained endurance subjects, only the one hour post-exercise VO\textsubscript{2} for the 80 minute at 50% of VO\textsubscript{2} max trial was significantly greater than its time-matched control. They concluded that for exercise intensities 55% of VO\textsubscript{2} max and durations 3 hours, there was no sustained elevation in metabolism, introducing a concept of a threshold that has also been suggested by others (Molé, 1990). The 70% of VO\textsubscript{2} max trials showed that EPOC increased in a linear fashion with exercise duration, with a greater EPOC response post the 80-minute
duration trial compared to the 20 and 50-minute trials. Further, that EPOC was elevated up to eight hours post exercise. These findings were similar to those reported by Bahr et al (1987) and Mahlum et al (1986), using similar protocols.

Based on the above findings, it appears that the magnitude and duration of the disturbance of pre-exercise metabolism, as reflected by an elevation in post-exercise oxygen consumption, is determined by the combination of the duration and intensity of aerobic exercise and can range from less than one hour to more than 12 hours.

### 2.2.2. Strength Exercise and EPOC

Compared to the effects of endurance exercise on EPOC, there have been fewer studies that have investigated the effect of resistance training on EPOC and energy expenditure post-exercise (Burleson et al., 1989; Elliot, Goldberg, & Kuehl, 1992; Melby, Tincknell, & Schmidt, 1992; Murphy & Schwarzkopf, 1992; Thornton & Potteiger, 2002). In one of the first studies to investigate EPOC following resistance training, Burleson et al (1989) found that recovery oxygen uptake (VO₂) was significantly higher during the first 30 minutes following a 27-minute circuit weight training session compared to the same intensity level of a 27-minute bout of treadmill running (activities performed at matched VO₂). However, the difference in EPOC between the two modes of exercise was not found at 60 and 90 minutes post-exercise. Whilst Burleson et al (1989) did not undertake separate control conditions for the determination of baseline values for EPOC, their data did suggest that weight training exercise may produce a greater elevation in EPOC then levels of steady state exercise (Melby et al., 1992).

Burleson’s et al (1989) findings were later substantiated in a study by Elliot et al (1992) who compared 40 minutes of cycling, heavy resistance training and circuit training. All forms of exercise experienced an increase in EPOC but only the resistance training sessions recorded significant post 30-minute results. However, there was no significant difference at 60 and 90 minutes post-exercise. Murphy and Schwarzkopf (1992) also compared standard weight training and circuit weight training on EPOC. Their results showed that the magnitude and duration of EPOC produced by circuit weight training was significantly greater than that produced by standard weight training. Elliot et al (1992) attributed the difference in results between the types of exercise to the amount of exercising skeletal muscle mass whilst Murphy
and Schwarzkopf (1992) attributed their findings to the intensity of exercise and the time duration of the exercise and rest components. These findings suggest that the intensity, duration and type of resistance training influence the extent of EPOC.

Whilst the above research suggests that the intensity of strength training produces different effects on EPOC, some caution must be used with respect to these results because of differences in the amount of work completed. In a recent study by Thornton and Potteiger (2002) that compared equal volumes of work for high- and low-intensity strength training sessions, support for the above concept was found. Fourteen trained subjects completed nine exercises consisting of two sets of eight repetitions at 85% of their eight repetition maximum (8 RM) for the high-intensity session and two sets of 15 repetitions at 45% of their 8 RM for the low intensity session. The results indicated EPOC was greater at 0-20, 45-60 and 105-120 minutes post-exercise for the high-intensity session compared to the low-intensity session. This evidence suggests that for strength training equated in terms of work volume, high-intensity exercise produces a greater EPOC magnitude and volume than low-intensity exercise.

The study by Thorton and Potteiger (2002) suggested that EPOC was still elevated two hours post-training but did not indicate when it had returned to baseline levels. In a study by Schuenke, Mikat and McBride (2002), the effect of 31 minutes of high-intensity strength training on EPOC, consisting of four circuits of bench press, power cleans and squats was investigated for a period of 48 hours post-training. The seven strength-trained males completed sets to failure at 10 RM intensity. The results indicated that EPOC was significantly elevated above baseline values immediately as well as at 14, 19 and 38 hours post-training.

A large variation has been reported in the literature for the duration of EPOC following strength training, as was the case with endurance exercise, with some reporting periods less than one hour (Elliot et al., 1992; Haltom et al., 1999; Melby et al., 1992; Murphy & Schwarzkopf, 1992) whilst others have reported periods greater than 12 hours (Dolezal et al., 2000; Gillette, Bullough, & Melby, 1994; Schuenke et al., 2002). The evidence from these investigations into the effect of strength training on EPOC suggests that the intensity of strength training is an important factor in determining the magnitude and duration of the recovery period post-exercise (Schuenke et al., 2002). It is also worth noting that the technical
skill of the lifter and the level of weight training experience have also been thought to influence the magnitude and duration of EPOC, as a more well-trained weight lifter may perturb homeostasis less owing to greater training adaptations and to more efficient lifting techniques than a lesser well-trained lifter (Melby et al., 1992; Thornton & Potteiger, 2002). A similar finding has also been found with respect to the comparison the recovery rates of endurance trained versus untrained individuals from the same relative and absolute work rates, with endurance-trained individuals recovering faster than the untrained (Short & Sedlock, 1997). The investigators attributed this finding to the trained individuals having a faster regulation of post-exercise metabolism as well as to a lesser stress resulting from the exercise stimulus than that incurred by the untrained individuals.

The determination of the impact on EPOC and associated physiological responses post-exercise between endurance training and strength are problematic due to differences in the type of exercise (continuous versus intermittent). However, investigators have suggested that strength training may produce a greater EPOC response than endurance training when equated for energy expenditure due to a greater physiological stress during the strength training, the result of higher exercise intensity (Gillette et al., 1994; Thornton & Potteiger, 2002) or increased working muscle mass (i.e. increased motor unit recruitment) (Elliot et al., 1992; Thornton & Potteiger, 2002).

Based on the above evidence, it is clear that the optimal recovery time before the next training session will depend on the type (strength or endurance) and intensity (high, medium or low) of training, with exercise such as high-intensity strength training, requiring a greater recovery time period than low-intensity aerobic endurance training of equal duration or energy expenditure (Bompa, 1994; Brooks et al., 2000; Elliot et al., 1992; Thornton & Potteiger, 2002). The practical implications of this are that the performance of another training session in the hours following an initial training session of either the same or different type may be adversely affected if sufficient recovery has not taken place. If an athlete is still in the process of recovering then there will be an increase in energy expenditure both during and in the recovery from the second bout of exercise at a given exercise intensity. This increase in energy expenditure during exercise would mean a reduction in mechanical efficiency, as any process that results in an increase in oxygen consumption without a corresponding increase in work output causes a reduction in efficiency (Kang et al., 1997). This increased oxygen cost for a given workload would also lead to a decrease in performance capacity (Bahr, 1992). The
other practical implication of insufficient recovery between training sessions is that if heart rate is the criteria used to set exercise intensity, then an elevated heart rate following the first training session, could mean that the intensity of subsequent training sessions are conducted above or below the designated level.

Bahr and colleagues (1991) highlighted the implications of insufficient recovery between exercise sessions in a study of resting oxygen consumption, net mechanical efficiency during sub-maximal cycling exercise and EPOC after continuous simulated combat exercise compared to a control trial. They reported an increase in oxygen consumption of 14 and 19% at rest and during the sub-maximal cycling respectively, and a reduction in net efficiency from ~25% to ~21%, compared to the control trial. In addition, they also reported an increase in mean heart rate and skin temperature of ~14 b.min\(^{-1}\) and ~1.4°C during the sub-maximal cycling exercise, respectively. Whilst this example was outside the scope of normal strength and endurance training protocols, it did emphasise that without an adequate recovery the subsequent exercise performances can be affected. In light of the above, consideration must be given to the timing of a second bout of exercise when designing training regimes where multiple training sessions are completed within the same day or on adjacent days. Also to the intensity and duration of the respective training sessions.

### 2.3. Physiological Adaptations to Strength and Endurance Training

The scope of literature in reference to strength and endurance training is beyond the scope of this thesis. Consequently, the following is a brief overview highlighting the central adaptations to strength and endurance training.

The adaptations that occur as a result of strength training have been well investigated (Costill, Coyle, Fink, Lesmes, & Witzmann, 1979; Delorme, 1945; Eyster, 1927). Strength training typically induces increased synthesis of myofibrillar proteins, which in turn results in increased muscle hypertrophy particularly in type II fibres (Hather et al., 1991; MacDougall et al., 1984; Staron et al., 1994; Staron et al., 1991; Tesch, 1987). There is also an alteration of the ratio of type II fibres (percentage of type IIa increases and type IIb decreases) (Adams et al., 1993; Fleck & Kraemer, 1988; Kraemer, 2000a; Kraemer, Fleck, & Evans, 1996; Staron et al., 1994; Staron et al., 1991). In addition to changes in muscle fibre size and type, there is also a reduction in mitochondrial volume and density (Fleck & Kraemer, 1988; MacDougall
et al., 1979; Tesch, 1987) as well as alterations in the activity of enzymes associated with aerobic energy pathways (Fleck & Kraemer, 1988; Tesch, 1987). A summary of the adaptations that occur as a result of long-term exposure to strength training is outlined in Table 2.1.

The degree of strength adaptation has been reported to be dependent on the type of resistance training undertaken, that is, whether the training is strength, power or endurance orientated (McDonagh & Davies, 1984; Wilson, 1993b, 1998a). Each type of resistance training imposes a different stimulus upon the body and therefore, a different level of adaptation in accordance with the continuum of strength qualities (Figure 2.3). Hence a resistance training regime that entails high resistance (high-intensity) with few repetitions develops maximum strength (the left side of the continuum) whilst, a low resistance (low-intensity) repeated many times develops strength endurance (the right side of the continuum).

![Figure 2.3. Theoretical continuum of strength qualities (Adapted from Fleck and Kraemer (1987)).](image)

In contrast to the adaptations that occur with strength training, endurance training adaptations are in most cases, the opposite (Hickson, 1980; Holloszy & Coyle, 1984; Sale et al., 1990a). Endurance training stimulates aerobic adaptation whilst strength training increases muscular strength and anaerobic adaptation (Tanaka & Swensen, 1998). Endurance training typically increases aerobic capacity via changes in respiratory capacity, increased mitochondrial and capillary densities and increased enzyme activity (Holloszy & Coyle, 1984; Kraemer, 2000a; Terjung, 1995). A summary of the adaptations that occur as a result of long-term exposure to endurance training are also provided in Table 2.1.
Table 2.1. Physiological adaptations that occur in response to strength and endurance training {Adapted from (Abernethy, Thayer, & Taylor, 1990; Fleck & Kraemer, 1997; Holloszy & Coyle, 1984; Kraemer, 2000a; Terjung, 1995)}.

<table>
<thead>
<tr>
<th>System Variable</th>
<th>Strength Training</th>
<th>Endurance Training</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle Fibres</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>Equivocal</td>
<td>Equivocal</td>
</tr>
<tr>
<td>Size (hypertrophy)</td>
<td>↑</td>
<td>Selective ↑</td>
</tr>
<tr>
<td>Type</td>
<td>Ila ↑, Ilb ↓</td>
<td>Ilb → Ila → I capacity</td>
</tr>
<tr>
<td><strong>Capillary Density</strong></td>
<td>NC or ↓</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Mitochondrial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Density</td>
<td>↓</td>
<td>↑</td>
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<tr>
<td><strong>Enzymes</strong></td>
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</tr>
<tr>
<td>Creatine Phosphokinase</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Myokinase</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td><strong>Enzymes of Glycolysis</strong></td>
<td></td>
<td></td>
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<tr>
<td>Phosphofructokinase</td>
<td>↑</td>
<td>Variable</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>NC or variable</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Intramuscular Fuel Stores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Phosphocreatine</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Glycogen</td>
<td>NC or ↑</td>
<td>↑</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Unknown</td>
<td>↑</td>
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<tr>
<td><strong>Maximal Oxygen Uptake</strong></td>
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<tr>
<td>(mL.kg⁻¹.min⁻¹)</td>
<td>NC</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
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</tr>
<tr>
<td>Heart rate</td>
<td>NC or ↓</td>
<td>↓</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>NC or ↓</td>
<td>NC or ↓</td>
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<tr>
<td>Diastolic blood pressure</td>
<td>NC or ↓</td>
<td>NC or ↓</td>
</tr>
<tr>
<td>Stroke Volume</td>
<td>NC or ↑</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Cardia Morphology – Left</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness</td>
<td>↑</td>
<td>NC</td>
</tr>
<tr>
<td>Chamber volume</td>
<td>NC</td>
<td>↑</td>
</tr>
</tbody>
</table>

NC = no change, ↑ = increase, ↓ = decrease, → = change.
In view of the differences in adaptation that occur with respect to strength and endurance training (Table 2.1), controversy exists as to the compatibility of training for both strength and endurance within the same training regime (Bell et al., 2000; Kraemer et al., 1995; Tanaka & Swensen, 1998), a concept that has been termed concurrent training (Hickson, 1980; Sale et al., 1990a).

2.4. Concurrent Training

Two hypotheses were put forward by Craig, Lucas, Pohlman and Stelling (1991) to explain why concurrent training may not be beneficial, as cited in a review of concurrent strength and endurance training by Leveritt, Abernethy, Barry and Logan (1999). The first hypothesis considered the chronic adaptations whilst the second considered the acute responses that occur with training for strength and endurance.

**Chronic Hypothesis** – “skeletal muscle cannot adapt metabolically or morphologically to both strength and endurance training simultaneously”.

**Acute Hypothesis** – “residual fatigue from the endurance component of concurrent training compromises the ability to develop tension during the strength element of concurrent training”.

The chronic hypothesis was attributed to the fact that many of the adaptations that occur at the muscle level in response to strength training are different to those found with endurance training. Whilst the acute hypothesis was attributed to the proposed reduction in the quality of strength training due to fatigue following endurance training, which would lead to a reduction in strength development over time.

A number of studies (Bell et al., 2000; Craig et al., 1991; Dolezal & Potteiger, 1998; Dudley & Djamil, 1985; Hickson, 1980; Hortobágyi et al., 1991; Kraemer et al., 1995; Sale et al., 1990b) have investigated the effects of combined strength and endurance training on the level of strength and endurance adaptation. The common trend with the majority of the research into the effects of concurrent training to date, is that there is interference in the development of strength and/or endurance qualities using concurrent training regimes compared to single mode strength and endurance training (Leveritt et al., 1999).
2.4.1. Effect of Concurrent Training on Strength Adaptation

One of the first studies to investigate the effects of combining strength and endurance training was undertaken by Hickson (1980), who compared a strength (S) group, an endurance (E) group and a combined strength and endurance (COMB) group over ten weeks of training. The S group performed leg strength exercises (load > 80% of one repetition maximum, 1 RM) five days/week, the E group performed 40 minutes of interval cycling and continuous running 6 day/week, and the COMB group combined routines of the S and E groups. Over the first seven weeks of training, the rate of strength improvement (measured by improvement in squat 1 RM) in the COMB group was similar to that of the S group (approximately 34%). However, this rate of improvement levelled off and declined during the final three weeks of training (approximately 25% improvement over starting values) for the COMB group whilst the S group recorded an improvement of 44%, and the E group no change. Hickson (1980) suggested that for the COMB group there was an upper limit to the development of strength, with aerobic endurance training inhibiting or interfering with further increases in strength development. He initially thought the result was due to the development of residual fatigue from the endurance training. However, he felt that this argument was inconclusive because the work performed per week on the cycle ergometer increased at approximately the same rate for both the E and COMB groups during the final weeks of the study. Hickson (1980) suggested that the effects of COMB training on strength development might be selective.

Kraemer et al (1995) used a similar design to Hickson (1980) to also examine the compatibility of combined S and E training using 35 physically active soldiers. The subjects were randomly assigned to four training groups: a COMB group, a COMB group that performed E training and upper body only S training (U/COMB), and whole body E and S groups. The S group completed a four day/week split routine of various lower/upper body exercises at a high-intensity (5 x 5 RM and 3 x 10 RM). The E group ran at an intensity greater than 80% of VO$_2$ max, consisting of continuous and interval training, while the COMB completed four days/week of the same sessions as the single S and E groups. The U/COMB completed the same E and S training sessions as the COMB group except that the subjects performed only the upper body S training exercises. The improvement in leg strength, as measured by a 1 RM leg-press, was greater for the S group (approximately 30%) compared to the COMB group (19.5%), which were greater than those of the U/COMB (9.6%) and E (1.7%) groups. Kraemer et al (1995) suggested that the simultaneous training
for S and E produced smaller strength gains than the strength training alone and like Hickson’s (1980) initial reasoning, suggested that the incompatibility of COMB training may be due to fatigue.

The impedance of strength development whilst concurrently undertaking E training has also been found by other investigators (Bell et al., 2000; Craig et al., 1991; Dolezal & Potteiger, 1998; Dudley & Djamil, 1985; Hortobágyi et al., 1991; Sale et al., 1990b), using a variety of training variables (Table 2.2). It must be pointed out though, that there have also been investigations (Abernethy & Quigley, 1993; Gravelle & Blessing, 2000; McCarthy, Agre, Graf, Pozniak, & Vailas, 1995; Sale et al., 1990a) that have reported no impedance of strength development with COMB training. Sale et al (1990a) in a 22-week training study had two groups undertake different S and E training routines. One group completed S training on one leg and a COMB training routine on the other leg. The other group completed E training on one leg and a COMB training routine on the other leg. The S session consisted of six sets of 15-20 repetitions of leg-press whilst the E session consisted of five times three-minute bouts of cycling at 90-100% of VO₂ max. The COMB session required both the S and E routines to be completed. Leg-press 1 RM increased in both the S and COMB groups with no significant differences between the S and COMB groups with respect to the pre to post increases, as too the muscle cross sectional area. Whilst Sale et al (1990a) concluded that concurrent S and E training did not interfere with S development when compared to S training alone, some caution must be used in interpreting the findings, as the results may have been influenced in part by the “central effect” of the training of the other leg, commonly referred to as the “cross over effect” or “cross education” (Cannon & Cafarelli, 1987; Hortobágyi, Lambert, & Hill, 1997). The cross education effect has been reported to induce strength improvements by as much as 77% or greater in the inactive limb musculature (Hortobágyi et al., 1997).
Table 2.2. Concurrent training studies where strength development has been impeded (S = strength, E = endurance, COMB = concurrent strength and endurance training, VO₂ max = maximal oxygen uptake, RM = repetition maximum).

<table>
<thead>
<tr>
<th>Author</th>
<th>Protocol Design</th>
</tr>
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<tbody>
<tr>
<td>(Bell et al., 2000)</td>
<td><strong>Four groups</strong>: S, E, COMB and control. S and E groups trained three day/week. S = upper/lower body resistance exercises. E = 30-42 minutes of cycling. COMB = completed both S and E routines but over six days/week. All groups trained for 12 weeks.</td>
</tr>
<tr>
<td>(Craig et al., 1991)</td>
<td><strong>Three groups</strong>: S, E and COMB. All groups trained three days/week for ten weeks. S = various upper/lower body exercises. E = running up to 35 minutes at 75% of heart rate max. COMB = completed both S and E routines on the same day with E immediately before S training.</td>
</tr>
<tr>
<td>(Dolezal &amp; Potteiger, 1998)</td>
<td><strong>Three groups</strong>: S, E and COMB. All groups trained three days/week for ten weeks. S = various upper/lower body exercises. E = running up to 40 minutes at 85% of heart rate max. COMB = completed both S and E routines on the same day.</td>
</tr>
<tr>
<td>(Dudley &amp; Djamil, 1985)</td>
<td><strong>Three groups</strong>: S, E and COMB. S = 2 x 30 second sets of maximal knee extension. E = 5 x 5 minutes of cycling at 40-100% of VO₂ max. S and E groups trained three days/week. COMB = same training as S and E groups but on alternate days. All groups trained for seven weeks.</td>
</tr>
<tr>
<td>(Hortobágyi et al., 1991)</td>
<td><strong>Three groups</strong>: S low-resistance, S high-resistance and control. Both low- and high-resistance = 40 minutes of circuit training with upper/lower body exercises plus two mile run, three days/week for 13 weeks.</td>
</tr>
<tr>
<td>(Sale et al., 1990b)</td>
<td><strong>Two groups</strong>: Group one = S and E on alternate days, Group two = S and E on same day. Group one = two days/week S, two day/week E, Group two = two days/week with S and E in single session. E = 6-8 x 3 minute bouts of cycling at 90-100% of VO₂ max. S = 6 x 15-20 RM of leg-press. Both groups trained for 20 weeks.</td>
</tr>
</tbody>
</table>

Both McCarthy et al (1995) and Abernethy and Quigley (1993) found no interference in strength development with COMB training compared to single mode training. McCarthy et al (1995) attributed their results to the reduced frequency of COMB training days used in their study, that is, three days/week compared to five to six days/week as used in other studies (Dudley & Djamil, 1985; Hickson, 1980), whilst Abernethy and Quigley (1993) attributed
their results to a different pattern of strength development in the triceps to that of the quadriceps (as used in other studies) during COMB training.

In view of the above evidence, the available literature is inconclusive as to the effect of COMB training on the development of strength compared to single mode strength training. However, as outlined earlier, the common trend is that there is interference in the development of strength qualities using COMB training regimes compared to single mode strength training. Further research is required before a more conclusive decision can be made regarding the effect of COMB training on strength development adaptations.

2.4.2. Effect of Prior Endurance Training on the Acute Strength Response

The above section outlined the strength adaptations that occur as a result of COMB training over a number of weeks (7-22). However, the acute strength response to a single bout of endurance training was not addressed.

The acute effects of endurance activity of varying time durations (Baker, Kostov, Miller, & Weiner, 1993) and modes of exercise (Abernethy, 1993; Forsberg, Tesch, & Karlsson, 1979; Lepers, Hausswirth, Maffiuletti, Brisswalter, & van Hoecke, 2000; Sahlin & Seger, 1995; Sherman et al., 1984) on the acute strength response have been investigated. Forsberg et al (1979) first reported that there were changes in muscular strength after a prolonged 60-mile endurance ski race and that there was a pronounced decrease (28%) in muscle strength in slow isokinetic contractions following endurance exercise. They attributed part of the reduction in muscle strength to their subjects’ level of dehydration post-race. In subsequent studies by Sherman et al (1984) and Sahlin and Seger (1995) similar findings were also reported.

Sherman et al (1984) reported that isokinetic leg-extension maximal peak torque (MPT) was significantly reduced (30-40%) 15-20 minutes following a 42.2 km marathon run. Even though MPT improved over subsequent days, the level of MPT remained less than pre-marathon MPT values for up to five to seven days post-marathon. Like Forsberg’s et al (1979) subjects, Sherman’s et al (1984) subjects also experienced dehydration and an acute loss of muscle strength but since the subjects’ hydration levels returned to pre-marathon levels by one day post-marathon yet reduced MPT values were still experienced, it appeared that acute dehydration could not account for the subjects’ loss of muscle function. The investigators
attributed the decline in MPT post-marathon to muscle glycogen depletion but over the following one to seven days, muscle glycogen levels returned to normal levels but reduced MPT values were still recorded. Therefore, Sherman et al (1984) suggested that muscle glycogen levels could not account for the reduced MPT values directly immediately or during the days post-marathon.

Sahlin and Seger (1995) also reported that maximal voluntary isometric force decreased to a mean 91% of the pre-exercise values after five minutes of exercise, 82% after 40 minutes and 66% at exhaustion, following a prolonged cycle to exhaustion at 75% of VO$_2$ max, an overall decrease of 34% and similar to the values reported by Sherman et al (1984). Further, Sahlin and Seger (1995) also reported that after 30 minutes post-exercise maximal voluntary isometric force had recovered to 80% of the pre-exercise value. The investigators suggested that the recovery of maximal voluntary force after prolonged endurance exercise occurs in two phases, where the initial phase is relatively short (a half time of a few minutes) whilst the second phase may have a half time of days or weeks, a point consistent with the findings of Sherman et al (1984). The investigators attributed the first phase of recovery to the restoration of muscle energetics or electrolyte balance whereas the second phase was related to the damage of structural elements of the muscles involved or to non-metabolic factors.

In a similar study to that of Sahlin and Seger (1995), Bentley, Zhou and Davie (1998) also reported significant reductions in isokinetic peak torque at 60$^\circ$.s$^{-1}$ after six hours of recovery from 30 minutes of high-intensity cycling exercise. However, unlike Sahlin and Seger (1995), Bentley et al (1998) reported that isokinetic peak torque values had returned to pre-test levels by 24 hours post-exercise. Whilst the same mode of exercise (cycling) was used by Sahlin and Seger (1995) and Bentley et al (1998), there was a notable difference in the time duration of the exercise undertaken, Sahlin and Seger (1995) ~85 minutes and Bentley et al (1998) ~30 minutes, a factor that has been shown to play a major part in the rate and level of recovery post-exercise (Baker et al., 1993).

Consequently, it would appear from the above literature that the mode of exercise (running, cycling, skiing etc.), the type of contraction (concentric only – cycling, concentric/eccentric – running) and the duration of the prior endurance activity might determine the acute level of strength response post-exercise. It would also appear that dehydration and muscle glycogen
depletion, whilst contributing factors in the interim period post-exercise, are not the major contributing source for the reduced maximal voluntary force levels post-endurance exercise.

2.4.3. Effect of Concurrent Training on Endurance Adaptation

Whilst it has been documented that strength improvements maybe compromised by COMB training (Hickson, 1980; Kraemer et al., 1995), the available literature also suggests that improvements in endurance adaptation are compromised by COMB training when compared to endurance training alone (Dolezal & Potteiger, 1998; Gravelle & Blessing, 2000; Kraemer et al., 1995; Nelson et al., 1990).

Dolezal and Potteiger (1998) compared the effect of COMB training on basal metabolic rate, 1 RM strength (squat and bench press) and VO$_2$ max over a ten-week training period. The subjects were randomly allocated to one of three training groups: E group, S group and COMB group. All groups trained three days/week with the COMB group completing both the S and E sessions on the same day, and the S training completed first. The COMB group’s VO$_2$ max increased 7% compared to the E trained group’s 13% improvement whilst the improvements in squat and bench press 1 RM were greater in the S group (24 and 23%, respectively) compared with the COMB group (19 and 12%, respectively). The E group recorded no change in strength values. The results indicated that the improvement in VO$_2$ max was compromised more than the improvements in lower body strength. A finding that was the reverse to other investigations (Dudley & Djamil, 1985; Hickson, 1980), which have reported a greater impedance of strength development than endurance development when incorporating COMB training programmes. Dolezal and Potteiger (1998) attributed their finding to the interferences found in strength training adaptations such as muscle fibre hypertrophy and increases in contractile proteins with associated decreases in capillary and mitochondrial densities.

Nelson et al (1990) also reported compromised endurance development for a COMB (isokinetic strength and bicycle ergometer) training group compared to an E (bicycle ergometer) trained only group over 20 weeks of training. However, unlike Dolezal and Potteiger (1998), Nelson et al (1990) reported that after 11 weeks of training both the E and COMB groups had similar gains in VO$_2$ max but during the last nine weeks of the study, the E group experienced significantly greater gains in VO$_2$ max compared to the COMB group. In
addition, the E group had a significant increase in citrate synthase activity whereas the COMB group did not. The investigators suggested that, “simultaneous training may inhibit the normal adaptation to either training program when performed alone”. Further, the level of interference may depend on the nature and intensity of the respective S and E training programmes. Similar to Dolezal and Potteiger (1998) and Nelson et al (1990), Gravelle and Blessing (2000) and Kraemer et al (1995) also reported a lower percent increase in VO$_2$ max for a COMB group compared to an E only group.

Unlike the above studies that compared the results to an E only group, Gravelle and Blessing (2000) compared the level of physiological adaptation to an alternate sequence of training. That is, they compared a COMB group that completed training in the order endurance then strength to a COMB group that completed the training in the reverse order. After 11 weeks of training consisting of three days per week involving 45 minutes of 5-6 lower body exercises designed to increase strength and 45 minutes of rowing at 70% of VO$_2$ max, three times per week, it was found that whilst there was no negative effects on strength development (assessed by changes in 1 RM leg-press), aerobic capacity had been impeded for the sequence endurance prior to strength. This suggests that sequence of training may be a factor in determining whether strength and endurance adaptations are optimised in a concurrent training programme, an issue that will be addressed later in this review.

In contrast to the above, there have also been a number of studies which have reported no interference in E development with COMB training compared to E training alone (Bell et al., 2000; Dudley & Djamil, 1985; Hickson, 1980; McCarthy et al., 1995). However, some caution must used in interpreting this finding, as the majority of these studies have used untrained subjects that have simultaneously started strength and endurance training, a factor which may alter the level of adaptation compared to either already strength or endurance trained subjects (Tanaka & Swensen, 1998).

In studies that have added a strength training component to already endurance-trained subjects to determine if strength training could improve endurance performance, the findings are inconclusive. Some studies (Hickson, Dvorak, Gorostiaga, Kurowski, & Foster, 1988) indicate improvements in performance whilst others have not (Bishop, Jenkins, Mackinnon, McEniery, & Carey, 1999; Tanaka et al., 1993). One such study was undertaken by Hickson et al (1988), who examined the impact of adding heavy resistance training on running and
cycling training in already endurance-trained cyclists and runners. The addition of three
days/week for ten weeks of leg strength exercises resulted in an improvement of 30% in leg
strength, 11% and 13% increase in cycling and running short term endurance (4-8 minutes),
respectively, and an increase in cycling time to exhaustion at 80% of VO₂ max from 71-85
minutes (no significant change in absolute VO₂ max was observed). The investigators
indicated that the adding of heavy resistance training to ongoing endurance training did not
result in negative performance effects. However, the inclusion of the strength training did not
significantly improve performance during a timed 10 km run.

In a similar study to that described by Hickson et al (1988), Bishop et al (1999) examined the
effects of resistance training on cycling endurance performance of 21 female cyclists. The
subjects were assigned to either an E only group (control) or a COMB group for 12 weeks of
training. The COMB group maintained normal weekly road cycling mileage and included
resistance training (two days/week) consisting of five sets 2-8 RM of parallel squats. The E
group maintained normal weekly road cycling mileage. The results indicated that the COMB
group experienced a significant increase in 1 RM squat (35.9%) over the 12 weeks. However,
unlike Hickson et al’s (1988) findings of an increase in time to exhaustion, the increase in leg
strength did not result in a significant change in performance (average power output during a
one hour cycle test).

A similar result was also observed in a swimming training study by Tanaka et al (1993),
where an increase in upper body strength in trained swimmers via adding dry-land resistance
training to the training regime, did not result in improved swimming performance. Whilst the
effects of COMB training, in the form of in-water resistance and endurance training on
swimming endurance performance have not been investigated (Tanaka & Swensen, 1998),
evidence exists that indicates that swim resistance training (in-water) improves a swimmer’s
velocity over distances up to 200 metres (Toussaint & Vervoorn, 1990) as well as improving
stroke force and distance per stroke (Tanaka et al., 1993; Toussaint & Vervoorn, 1990). This
suggests that the modality of resistance training may play a greater part in improving the
compatibility of resistance and endurance training than using traditional resistance training.
However, further research is required across other types of endurance sports such as cycling
and running before the extent of compatibility of sport-specific resistance training and
endurance training can be known.
2.4.4. Effect of Prior Strength Training on the Acute Endurance Response

The effect of resistance training on the acute strength response post-exercise is well documented (Gibala, MacDougall, Tarnopolsky, Stauber, & Elorriaga, 1995; Häkkinen, 1992; Häkkinen, Pakarinen, Alén, Kauhanen, & Komi, 1988; Linnamo, Häkkinen, & Komi, 1998). In studies that have investigated the effect of a single bout of resistance exercise on the acute strength response, measured as a maximal isometric contraction, have reported that reductions in muscle function, whilst greatest immediately post-exercise (Gibala et al., 1995; Häkkinen, 1993; Linnamo et al., 1998), can persist for periods up to 96 hours (four days) post-exercise (Gibala et al., 1995; Sargeant & Dolan, 1987).

In an investigation of the time course of maximal force production following heavy leg-extension strength training consisting of five sets of 10 RM, Linnamo et al (1998) reported that maximal force production was reduced by greater than 20% immediately following exercise. Whilst force production levels had recovered slightly by two hours post-training, they were still substantially lower than pre-training levels (>15%) and took two days to return to the pre-training state. Linnamo et al (1998) finding supported an earlier finding by Häkkinen (1993) who investigated neuromuscular fatigue and recovery in male and female athletes during and following heavy resistance exercise. Häkkinen (1993) reported that after 20 repetitions of squat 1 RM there was a reduction of approximately 24 and 20% respectively in males and females bilateral isometric force immediately post-exercise and a significant decrease in maximal integrated electromyography (iEMG) which were still below pre-training levels two days post-training. These findings indicated that high-intensity resistance training might result in acute fatigue leading to a decreased force production capacity in subsequent activities undertaken within close proximity, time wise, to the strength training session.

To date there is no empirical evidence regarding the acute effects of a single session of resistance training on subsequent endurance performance. However, in an investigation of the neuromuscular response of males and females to two successive strength training sessions in the one day using the same muscle groups, Häkkinen (1992) reported that whilst a decrease in maximal strength was observed from the beginning to the end of the first session, a greater decrease in maximal strength accompanied by a shift (worsening) in the force time curve occurred, was observed for the second session. Häkkinen (1992) suggested that this result, along with that of reduced iEMG data during the second session, indicated that acute fatigue
may occur not only in the contractile components of the examined muscles but also in the nervous system.

In light of the above findings by Häkkinen (1992), it may be that bouts of activity using the same muscle groups as those used previously in the day or the preceding days for training may reduce the performance capabilities of the muscle groups in subsequent activities. This line of reasoning is supported by a study (Gleeson et al., 1995) that examined whether exercise containing a large eccentric component (bench stepping) compared to exercise that did not (uphill walking) affected the physiological responses in subsequent dynamic exercise performed two days following the initial training exercise. Two days after the bench stepping, all subjects exhibited elevated levels of serum creatine kinase and plasma cortisol concentrations compared to the uphill walking. Further, respiratory rate, respiratory exchange ratio, heart rate, ratings of perceived exertion and venous blood lactate were all higher during cycling after the bench stepping compared to the uphill walking. The authors attributed their findings to the reduction in force generating capacity of damaged muscle fibres after the bench stepping which incurred a greater metabolic stress in a reduced number of undamaged active fibres during exercise. They also suggested that modified muscle fibre recruitment patterns, with an increased relative contribution to force production from glycolytic type II fibres may have contributed to the result. The authors concluded, “dynamic sub-maximal exercise performed two days after an exercise bout with a large eccentric component produces physiological responses that are indicative of a higher relative exercise stress”.

Whilst the above study by Gleeson et al (1995), did not involve resistance training, it does suggest that activities like resistance training which involve a large eccentric component may increase the physiological and metabolic responses experienced in subsequent endurance orientated exercise completed in close proximity to the bout of prior exercise. The significance of this is that the metabolic cost of performing subsequent endurance exercise may be increased, as highlighted in the study by (Bahr et al., 1991) outlined earlier.

2.5. The Effect of the Sequence of Concurrent Training on Strength and Endurance Adaptation

The majority of the studies outlined thus far in the literature review for concurrent training regimes, have utilised S and E groups and a combination of these two as the COMB group
and examined the level of strength and aerobic capacity adaptation over time. However, these studies have not attempted to address the issue of the sequence of performing the training in the COMB group or if the manipulation of the sequence of training alters the level of adaptation. To date only a small proportion of concurrent training studies (Bell, Petersen, Quinney, & Wenger, 1988; Bell et al., 1991b; Collins & Snow, 1993; Gravelle & Blessing, 2000) have investigated this issue.

In the first study of its type Bell et al (1988) investigated the effects of endurance and strength training in two different sequences on sub-maximal, VO2 max and strength acquisition. The premise was that completing the programs in a sequential program might avoid the incompatibility of training strength and endurance at the same time. This was due to the findings of a study by Riedy, Moore and Gollnick (1985) that showed extensively hypertrophied rodent muscle responded to training in a similar manner to that of normal muscle. Bell et al (1988) allocated 16 rowers to two groups with one group completing five weeks of endurance training prior to five weeks of strength training whilst the other group completed the reverse training order. The endurance training consisted of 60 minutes of rowing at an intensity equal to 85-90% of maximum heart rate, five days per week whilst the strength training consisted of 12 stations of variable high-velocity (3.0 rad.s⁻¹) resistance training for four sessions per week. The strength training required the completion of two intervals of 20 seconds exercise: 20 seconds rest with 60 seconds rest between stations. The results indicated that the sequence of training did influence the level of physiological adaptations to each program. The completion of the sequence endurance then strength training did not result in as high strength gains as the sequence strength then endurance, whilst VO2 max improved under both sequences.

In a later study by Bell et al (1991b) using a similar design to that described above but for low-velocity (1.05 rad.s⁻¹) resistance training instead of high-velocity resistance training and two intervals of 30 seconds exercise: 30 seconds rest instead of 20 second periods, a similar outcome was found, with the sequence of training altering the level of adaptation. The sequence of strength then endurance showed no significant decrease in peak torque or total work of the E training, suggesting that the maintenance of strength occurred compared to the endurance then strength sequence, which resulted in a loss of aerobic capacity improvements during the S phase.
The above studies (Bell et al., 1988; Bell et al., 1991b) utilised sequential programmes, however in a study by Collins and Snow (1993) the effects of completing strength and endurance training within the same training regime but in different sequences were investigated. The strength training consisted of ten exercises for two sets of 3-12 repetitions whilst the endurance training involved 20-25 minutes running at 60-90% of heart rate reserve. No significant difference was found between the two sequences as assessed by VO$_2$ max, 1 RM bench press, arm curl and leg-press strength after seven weeks of training. Based on Collins and Snow’s (1993) findings, one could assume that the development of strength and endurance are independent of the sequence of training. In contrast, in a more recent study by Gravelle and Blessing (2000), who compared the physiological responses of 19 trained women using two different training sequences to 11 weeks of concurrent strength and endurance training, it was found that the sequence of training did not influence strength training adaptations but appeared to limit aerobic adaptations. The authors used strength training consisting of 45 minutes of 5-6 lower body exercises designed to increase strength whilst the endurance training involved rowing three times per week for 45 minutes at 70% of VO$_2$ max. They reported that 1 RM leg-press was identical across the sequences strength immediately before endurance and endurance immediately before strength compared to a S only group at the end of the training period but that whilst VO$_2$ max increased for both sequences, the increase from pre- to post-training was not significant for the sequence endurance then strength.

In light of the above sequential studies by Bell et al (Bell et al., 1988; Bell et al., 1991b) and those studies comparing the various sequences of concurrent training (Collins & Snow, 1993; Gravelle & Blessing, 2000), as to the numerous studies comparing various training programmes (strength and endurance training on the same day and alternate days) with mixed results (Dudley & Djamil, 1985; Sale et al., 1990b), it is unlikely that the sequence of training is independent of the level of strength and endurance adaptation. Consequently, further research is required to determine the influence that the sequence of training has on the responses and adaptations to training. Furthermore, the scheduling of the training sessions needs to be investigated, that being what time interval should be allowed between training sessions, in order to avoid any residual effect of fatigue or other sources of interference.
2.6. Possible Mechanisms for Compromised Responses and Adaptations with Concurrent Training

A number of mechanisms thought responsible for the impedance of strength and/or endurance development, whilst undertaking concurrent training have been put forward by investigators. One of the first mechanisms proposed by early investigations (Hickson, 1980) as being responsible for the reduced level of strength development during concurrent training, was overtraining. However, more recently it has been suggested that this mechanism may not be a factor contributing to the impedance of strength and endurance development but this is still a debated issue within the literature (Dudley & Fleck, 1987; Leveritt et al., 1999).

The mechanisms more widely accepted as contributing to compromised strength and/or endurance development are changes in muscle fibre transformation and hypertrophy, alterations in endocrine responses and motor unit recruitment and residual fatigue (Chromiak & Mulvaney, 1990; Dudley & Fleck, 1987; Leveritt et al., 1999; Tanaka & Swensen, 1998). These mechanisms have been grouped by researchers into two major sources.

Chronic sources, which encompass

- changes in muscle fibre transformation and hypertrophy processes,
- altered endocrine responses, and
- alterations in motor unit recruitment patterns,

and an acute source, residual fatigue and those factors that may contribute to fatigue, such as neuromuscular fatigue, muscle damage and the depletion of muscle glycogen stores (Chromiak & Mulvaney, 1990; Leveritt et al., 1999).

2.6.1. Overtraining

Overtraining has been defined as “a decline or lack of improvement in physical performance, accompanied by underlying physiological changes resulting from a high volume and/or intensity of training without adequate recovery over a relatively long time period” (Chromiak & Mulvaney, 1990). In light of this definition and the common practice amongst concurrent training studies of having COMB groups undertake both routines of the S and E only groups,
it is conceivable that overtraining might be a factor that contributes to impeded strength and/or endurance development with concurrent training. For example, in the study by Hickson (1980), three groups (S, E and COMB group) completed ten weeks of training. The COMB group completed the same number of days of training as the S (five days) and E (six days) groups and the same routines but trained both on the same day. Therefore, increasing the COMB groups training volume without a reduction in the training intensity. Consequently, the increased volume and intensity of training for the COMB group compared to the S and E only groups may have provided insufficient recovery between training sessions. The accumulation of the inadequate recovery therefore may have resulted in overtraining and hence a reduced level of performance in subsequent training sessions compared to the S and E only groups.

However, the concept of overtraining being a mechanism responsible for compromised strength and/or endurance development has been questioned because few studies (Dolezal & Potteiger, 1998) have reported both strength and endurance impedance using a COMB group compared to S and E only groups (Leveritt et al., 1999). The majority of studies have reported the impedance of only one performance measure, either strength (Hickson, 1980; Hunter, Demment, & Miller, 1987; Kraemer et al., 1995) or endurance (Nelson et al., 1990). This was evident in the example given above (Hickson, 1980), for even though the COMB group completed both the S and E groups’ training frequency and routines, only strength development was reported to be impeded, as the endurance measure (VO₂ max) continued to improve over the weeks that the strength declined.

Support for other sources other than overtraining being responsible for the impedance of S and/or E development with concurrent training were provided in a study by Dudley and Djamil (1985). They, like Hickson (1980), used three training groups (S, E and COMB group) with the S group performing two sets of 30 seconds of maximal isokinetic knee extension three days per week, the E group five bouts of five minutes of cycling at 40-100% of VO₂ max three days per week and the COMB group the combined routines of the S and E groups but on alternate days. The volume of training per session of the COMB group by Dudley and Djamil’s (1985) subjects was considerably reduced compared to that of Hickson’s (1980) subjects, including the number of training sessions but like Hickson (1980) who reported reduced strength gains in the COMB group compared to the S group, Dudley and Djamil (1985) also reported a reduced magnitude of increases in angle specific torque. The
considerable difference in the total volume and frequency of training between the studies yet a similar outcome suggests that some factor other than overtraining might have been responsible for the impeded strength gains within the COMB trained group. For if overtraining was responsible, then both strength and endurance development should have been impeded when compared to the S and E only groups (Leveritt et al., 1999). “However, this argument presumes that the thresholds for the effects of overtraining to become apparent on strength and endurance measures are similar” (Leveritt et al., 1999). This may not be the case and requires further investigation before overtraining can be discounted as a mechanism responsible for the impedance of strength and endurance development in concurrent training.

2.6.2. Chronic Mechanisms

The chronic mechanisms proposed for the impedance of strength and endurance development were summarised by Craig et al (1991) in the hypothesis that suggested that “skeletal muscle cannot adapt metabolically or morphological to both strength and endurance training simultaneously”. When the physiological adaptations that occur in response to strength and endurance training are considered (Table 2.1), the concept of this hypothesis appears plausible. The direct research evidence to support this hypothesis is limited, as there are few studies (Bishop et al., 1999; Kraemer et al., 1995; Nelson et al., 1990) that have measured metabolically and/or morphological responses and adaptations that occur with concurrent training. However, based on the evidence obtained to date with respect to concurrent training and single mode strength and endurance studies, the following mechanisms have been identified as possible chronic sources of the impedance of strength and/or endurance development.

2.6.2.1. Transformation of Muscle Fibre Types

It is documented that during resistance training there is a transformation of type IIb fibres to type IIa, thereby altering the ratio of type II fibres (Abernethy, Jürimäe, Logan, Taylor, & Thayer, 1994; Adams et al., 1993; Fleck & Kraemer, 1997; Kraemer et al., 1996; Staron et al., 1994; Staron et al., 1991). Comparably, endurance training is also documented to produce a similar transformation of type II fibres (Chi et al., 1983; Fitts & Widrick, 1996; Jansson & Kaijser, 1977; Ryschon, 1994). It might be expected, given the similarity between fibre type transformations for both strength and endurance training that no interference in strength or
endurance development would be found if undertaken concurrently. This premise has been supported by studies (Nelson et al., 1990; Sale et al., 1990a) that have examined muscle fibre transformations following concurrent training compared to single mode strength and endurance training. Nelson et al (1990) and Sale et al (1990a) both reported that there was no difference in strength development and little difference in fibre type change between COMB and S trained groups over 20 and 22 weeks of training, respectively. In contrast to the results of these two previous studies, Kraemer et al (1995) reported that whilst no difference in fibre type transformation was found between COMB and S trained groups over 12 weeks of training, an impedance of strength development was found. It is thought that this impedance may be a function of neural mechanisms or unknown changes in the type IIa fibres (Kraemer, 2000a). To date the difference in findings with respect to strength development between the above studies has not been explained.

The studies by Nelson et al (1990), Kraemer et al (1995) and Sale et al (1990a) all used untrained subjects and reported similar levels of fibre transformation between COMB and S trained groups. However, in a study using trained female endurance cyclists that added 12 weeks of resistance training to their existing cycling endurance program, Bishop et al (1999) reported no change in the percentage of type IIa and type IIb fibres. The absence of a change in fibre composition was attributed to the cyclists having a limited potential for fibre type transformation, due to the small percentage of type IIb fibres (1.4%) compared to previous studies (Staron et al., 1994; Staron et al., 1991) involving female subjects (15% Type IIb fibres). Interestingly, Bishop et al (1999) reported an increase in 1RM strength but no change in average power output in a one-hour cycle test. This suggests that the level of muscle fibre transformation and hence level of strength and endurance development in concurrent training studies, may be dependent upon the existing training status and fibre composition level of the subjects.

Based on the limited evidence to date (Kraemer et al., 1995; Nelson et al., 1990; Sale et al., 1990a), concurrent training does not appear to limit the transition of muscle fibre types normally experienced with strength training in untrained subjects (Staron et al., 1994; Staron et al., 1991) but the extent to which the transformation impacts on strength development remains unknown due to the conflicting findings. Further, the impact of muscle fibre transformation on strength development in trained subjects warrants further investigation.
2.6.2.2. Muscle Fibre Hypertrophy

The adaptive response of a muscle depends on the type of training stimulus applied, as outlined earlier in this review. If the training stimulus is strength orientated, muscle adaptation can take the form of hypertrophy in which myofibres increase in size but retain their initial ultra-structural and biochemical properties (Williams & Neufer, 1996). The degree to which muscle hypertrophy occurs is dependent upon the difference between protein synthesis and degradation that is, the net protein synthesis (MacDougall et al., 1995). The rate of protein synthesis has been shown to increase within 3-24 hours post a bout of strength training (Chesley, MacDougall, Tarnopolsky, Atkinson, & Smith, 1992; MacDougall et al., 1995; Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997; Yarasheski, Zachwieja, & Bier, 1993) and remain elevated for up to 24 hours in trained athletes before returning to resting levels (Chesley et al., 1992; MacDougall et al., 1995).

It is thought that the pattern of expression of muscle myofibrillar protein may be regulated at the transcription and post-transcription levels of selected genes (Schiaffino & Reggiani, 1996). Therefore, the alterations in isoform expression following strength training may be due to proportionate changes in the quantity of the corresponding messenger ribonucleic acid (mRNA) (Periasamy, Gregory, Martin, & Stirewalt, 1989), with the quantity of mRNA differing according to the duration and/or intensity of the training stimulus (Williams & Neufer, 1996; Willoughby & Pelsue, 1998). The mechanisms by which muscle nuclei increase transcription of specific muscle mRNAs in response to strength training is not known. However, it has been proposed that the myogenic regulatory factors (MRFs), MyoD, myogenin, myf5 and MRF4, which are skeletal muscle specific transcription factors, may be important for the regulation of many myofibrillar genes (Baar, Blough, Dineen, & Esser, 1999; Florini, Ewton, & Coolican, 1996; Schiaffino & Reggiani, 1996). The results of a number of recent animal studies have revealed that MRF expression is elevated in response to either increases (Lowe, Lund, & Alway, 1998; Mozdziak, Greaser, & Schultz, 1998) or decreases (Delday & Maltin, 1997) in muscle activity with MyoD preferentially expressed in muscles composed of predominantly fast twitch fibres while myogenin is more abundant in muscles composed predominantly slow twitch fibres (Alway, Lowe, & Chen, 2001). Another factor also thought to be an important regulator is the mechano growth factor, insulin-like growth factor I (IGF-1) (Bamman et al., 2001; Goldspink, 1999) produced by active muscle
which has been found to control local tissue repair, maintenance and remodelling (Goldspink, 1999).

The outcome of the above processes is that resistance training evokes hypertrophy of both type I and type II fibres (Fleck & Kraemer, 1988, 1997; Kraemer, 2000a; Thorstensson, Hultén, von Döbeln, & Karlsson, 1976), whilst endurance training induces little or no change in the size of either type I or type II fibres (Kraemer, 2000a) with adaptation taking the form of remodelling, in which myofibres do not enlarge but acquire different enzymatic and structural characteristics, often accompanied by changes in microvasculature (Williams & Neufer, 1996). Even though the level of protein synthesis following endurance training has not been as extensively researched as resistance training (Rennie & Tipton, 2000), indications are that the levels of protein synthesis following endurance training are notably less than resistance training (Carraro et al., 1990; Tipton, Ferrando, Williams, & Wolfe, 1996). This difference in protein synthesis and hence muscle fibre type hypertrophy might be an underlying mechanism for the impedance of strength development during concurrent training when compared to strength training alone.

There is limited evidence concerning the changes that occur in muscle fibre types with combined strength and endurance training (Bell et al., 2000; Kraemer et al., 1995; Nelson et al., 1990), as was the case with muscle fibre transformation. However, Kraemer et al (1995) measured fibre size changes over 12 weeks of training and suggested that differential changes in fibre hypertrophy between COMB and S groups may contribute to the reduced strength development found with respect to concurrent training. Kraemer et al (1995) reported that the S group experienced hypertrophy of type I, IIa and IIc fibres, the E group atrophy of type I and IIc whilst the COMB group experienced hypertrophy in only type IIc fibres. The findings suggested that the combination of endurance and strength training produces smaller increases in muscle fibre areas and an explanation as to why improvements in leg strength were not found to be as great in the COMB group compared to the S group.

Tanaka and Swensen (1998) outlined in their review of the impact of resistance training on endurance performance, that the smaller muscle fibres and associated changes in myofibre contractile properties induced by endurance training (i.e. decreased maximum shortening velocity in type II fibres, increased maximal shortening velocity in type I fibres and reduced peak tension developed in all fibres) may facilitate aerobic processes while compromising
anaerobic power and muscle strength. This would help explain why Kraemer’s et al (1995) COMB trained subjects experienced similar increases in VO₂ max to that of the E group but reduced leg strength development when compared to the S group.

Nelson et al (1990), like Kraemer et al (1995), also reported differences in muscle fibre changes between S, E and COMB groups. However, unlike Kraemer et al (1995), Nelson et al (1990) reported significant hypertrophy of type I, IIa and IIb fibres in the COMB and E groups but only hypertrophy of type IIb fibres in the S group. Interestingly, strength development was not impeded in the COMB group but endurance development (VO₂ max) was over the duration of the study. This suggests that although concurrent training may disrupt muscle fibre hypertrophy patterns, other sources such as neural mechanisms and/or alterations in isoform expression may contribute to the maintenance of strength levels (Leveritt et al., 1999).

Even though there have been studies completed on the effects of strength (Carroll, Abernethy, Logan, Barber, & McEniery, 1998; Chesley et al., 1992; Staron et al., 1991; Willoughby & Pelsue, 2000; Yarasheski et al., 1993) and endurance training (Carraro et al., 1990; Tipton et al., 1996) on muscle protein synthesis post-training, there have been limited investigations of the effects of concurrent training on muscle protein synthesis (Tipton et al., 1996) or alterations in isoform expression in humans as a result of combined strength and endurance training (Psilander, 2002). However, the evidence from concurrent training studies using rodents (Riedy et al., 1985; Stone, Brannon, Haddad, Qin, & Baldwin, 1996) suggests that there may be interference at the level of gene expression for strength and/or endurance training adaptations when the two forms of training are combined.

Riedy et al (1985) examined the response of previously hypertrophied soleus and plantaris muscle of rats to endurance training over 13 weeks. It was found that extensively hypertrophied muscle responded to endurance training in a similar manner to that of muscle that was not hypertrophied. However, in a study by Stone et al (1996) who examined the adaptive responses of hypertrophying skeletal muscle to endurance training in 56 female Sprague-Dawley rats over a period of six weeks, it was found that the level of myosin heavy chain (MHC) isoform expression was altered according to the type of loading applied. This indicated that the degree of loading imposed on a fast-twitch muscle may be a critical factor in determining the relative pattern of fast MHC adaptation when repetitive types of
mechanical activity (running, cycling etc) are imposed on the limb musculature’. Interestingly, they also reported that increases in muscle oxidative enzyme content due to endurance training were also compromised when a hypertrophying process was occurring concurrently. Such findings have prompted some researchers (Allen, Monke, Talmadge, Roy, & Edgerton, 1995a) to postulate that adapting fibres may be limited in that they can only adequately support the expression of proteins for higher endurance capabilities (e.g. mitochondria) at the expense of high force-generating potential (contractile components) and vice-versa (Stone et al., 1996).

It is possible that the influencing mechanism in the reduced hypertrophy with concurrent training regimes is the time course between the strength and endurance training stimulus. As mentioned above, the rate of protein synthesis has been shown to increase within 3-24 hours after a bout of strength training (Chesley et al., 1992; MacDougall et al., 1995; Phillips et al., 1997; Yarasheski et al., 1993) and remains elevated for up to 24 hours in trained athletes before returning to resting levels (Chesley et al., 1992; MacDougall et al., 1995). Unfortunately, the time course of protein synthesis following endurance training has not been established (Rennie & Tipton, 2000). However, it is possible that the completion of an endurance training session prior to the completion of transcription from the strength training sessions being reached, may impede or alter the level of mRNA activity for specific genes associated with muscle hypertrophy such as MHC and MRFs, with the result being a reduced rate of protein synthesis and hypertrophy and hence a lower rate of strength development.

Similar to the increased rate of protein synthesis during the first 24 hours following strength training, it has been reported that the transcriptional time course of a number of metabolic genes following endurance-orientated exercise are also elevated for a similar time period (Pilegaard et al., 2002; Pilegaard, Ordway, Saltin, & Neufer, 2000). Therefore, the reverse process to that outlined earlier for strength training completed prior to endurance training may also apply to endurance training followed closely by a strength training session, with the mRNA activity of selected genes related to aerobic processes such as mitochondria proteins, being impeded. Thereby reducing the level of aerobic development over time. A concept that was alluded to by Dolezal and Potteiger (1998) after finding the improvement in VO2 max after ten weeks for a COMB group was compromised compared to an E group. However, there is no research to date to ratify this concept. A theoretical example of how this impedance process may work is provided in Figure 2.4.
Figure 2.4. A theoretical graphical representation of the possible altered kinetics of skeletal muscle mRNA up regulation for muscle hypertrophy following a combined strength and endurance training stimulus.

(A) The mRNA response of a gene associated with muscle hypertrophy following the application of an initial training stimulus. Whilst the majority of the evoked response is lost prior to the second stimulus application, the net increase from the proceeding session accumulates with each subsequent session. (B) The dark and light coloured columns represent the strength and endurance training mRNA responses, respectively. The completion of an endurance training session in the hours following the strength training session impedes the normal strength training mRNA response resulting in mRNA down regulation compared to (A). (C) The combined strength and endurance training, as indicated by the striped coloured columns, results in a reduced level of mRNA up regulation (middle dotted line) and hence a lower level of muscle hypertrophy compared to when the strength training is completed alone, represented by the dark coloured columns (top solid line) {Adapted from Williams and Neufer (1996)}.  

![Graph of mRNA response over training sessions](image-url)
Whilst the exact scenarios as described above have not been examined to date, an investigation of the possibility of altered gene expression of MHC, IGF-1 and MRFs following two sequences of concurrent training has been completed (Psilander, 2002). Four healthy male subjects completed two training sequences: (1) 45 minutes of endurance cycling at 70% of VO₂ max followed 15 minutes later by 20 minutes of heavy strength training, and (2) the same training protocols but in the reverse order. The strength training consisted of four sets of leg-press and -extension with each set to exhaustion (repetition range 6-14). Following each training sequence, muscle biopsies were taken immediately and 1, 2, 6, 24 and 48 hours post-training. No significant difference was found between the two training sequences for the majority of the time points for any of the genes, most likely due to the lack of subject numbers. However, it was reported that the sequence of strength then endurance training showed a greater mRNA concentration for the genes myogenin, MHC1 and MHCIIa across the time points 6-48 hours post-training compared to the sequence, endurance then strength. No real changes were found for IGF-1 for either training sequence. Even though the study used subjects who had not previously participated in strength or endurance training, which may account for some of the findings, the results do support the idea that the sequence and possibly the proximity in which concurrent training sessions are completed, may be important in determining the level of expression of genes associated with hypertrophy and hence strength development.
Due to the limited information available on protein synthesis and the possible alterations in gene expression for both muscle hypertrophy and aerobic responses in humans as a result of combined strength and endurance training, further research is required before this possible mechanism for impedance of strength and/or endurance adaptations is fully understood.

2.6.2.3. Alterations in Motor Unit Recruitment

The impedance of strength and/or endurance development during concurrent training cannot be wholly attributed to the inhibition of muscle fibre transformation or hypertrophy, and as such, alterations in motor unit recruitment are considered to be a contributing factor (Dudley & Fleck, 1987; Leveritt et al., 1999). To date there have been no concurrent training studies that have investigated this proposed source of interference in strength and endurance development. However, the existing knowledge available concerning recruitment patterns during strength (Sale, 1987; Wilson, 1994a) or endurance training (Burke, 1995; Ryschon, 1994; Sale, 1987) suggest that this mechanism may play a role in inhibiting the ability of concurrent training adaptations to reach the level of strength or endurance training alone adaptations.

Motor units are recruited in order according to their motoneuron size, referred to as the size principle, thus slow twitch motor units are recruited first followed by fast twitch intermediate and fast twitch motor units (Enoka, 1994; Kraemer, 2000b; Sale, 1987). The corresponding order of muscle fibre recruitment is from type I to type IIA to Type IIb (Enoka, 1994).

During strength training, athletes perform a number of repetitions based on their repetition maximum for a given exercise (i.e. the resistance that can only be lifted once, 1 RM), with the resistance varying depending on the strength quality being trained (i.e. maximum strength, power or strength endurance) (Fleck & Kraemer, 1987). For example, 85-95% of 1 RM for maximum strength gains whilst 30-50% of 1 RM for strength endurance capabilities (Feigenbaum & Pollock, 1997). Typically, high-intensity strength training using resistances below a 6 RM load requires the recruitment of a large proportion of available motor units (Kraemer, 2000a). As the number of repetitions progresses throughout the set, there is a corresponding greater activation of more motor units as the muscle fatigues (Sale, 1987; Wilson, 1994a), with all or near all motor units, both slow and fast twitch, being recruited during the final repetition as illustrated below (Figure 2.5) (Sale, 1987). In association with
the recruitment of more motor units as the muscle fatigues, there is also an increase in the firing rate of the motor units (Belanger & McComas, 1981; Häkkinen & Komi, 1986; Sale, 1987; Wilson, 1994a).

![Motor Unit Activation (%)](image)

**Figure 2.5.** A theoretical graphical representation of the number of motor units activated according to the number of repetitions completed {Adapted from Sale (1987)}.

During prolonged endurance exercise the recruitment pattern of motor units is notably different to that of the strength training scenario. Research has shown, using the glycogen depletion technique on samples taken from the vastus lateralis muscle, that the type of motor units recruited for cycling exercise changes as the intensity of exercise (expressed as a percentage of VO2 max) increases (Sale, 1987).

- Low-intensity exercise up to approximately 40% of VO2 max, predominantly slow twitch motor units (Type I fibres) are recruited (Vøllestad & Blom, 1985).
- Moderate-intensity exercise at 50-75% of VO2 max, both slow twitch (type I fibres) and fast twitch intermediate (type IIa fibres) motor units are recruited (Essén, 1978;
- High-intensity exercise above 90% of VO₂ max, near all motor units are recruited (Essén, 1978; Vøllestad & Blom, 1985).

It is also worth noting that the recruitment patterns of motor units are not uniform across exercise intensities but change as the intensity increases from moderate to high. At intensities below 90% of VO₂ max motor units are recruited in the order of slow twitch > fast twitch intermediate > fast twitch according to the size principle (Vøllestad & Blom, 1985; Vøllestad et al., 1984). However, for intensities above 90% of VO₂ max the reverse recruitment order applies (Gollnick, Armstrong, Sembrowich, Shepherd, & Saltin, 1973; Vøllestad & Blom, 1985). In addition, it has also been found that during low to moderate intensity exercise there is an increase in the recruitment of larger more powerful fast twitch motor units as fatigue begins to occur with exercise duration in order to maintain power output (Burke, 1995; Gollnick, Armstrong, Saubert et al., 1973; Ryschon, 1994). The firing rate of motor units during endurance exercise has also been found to be less than that experienced during strength training due to the lower level of force production required (Sale, 1987; Wilson, 1994a).

The above discussion indicates that motor units are recruited according to the intensity levels of exercise with slow twitch motor units predominantly recruited during low-intensity endurance exercise whilst a greater percentage of all motor units are recruited during maximal resistance exercise. Therefore, different motor unit recruitment patterns are utilised for the respective types of training (Sale, 1987). In view of this concept, it has been suggested that motor unit recruitment patterns may be altered when strength and endurance training are undertaken simultaneously, with concurrent training hindering the organisation of efficient motor unit recruitment patterns necessary for forceful muscular contractions at the level of peripheral or central nervous system (Chromiak & Mulvaney, 1990). This line of reasoning is supported by evidence that has shown that vertical jump ability of endurance trained athletes decreases with endurance training (Ono, Miyashita, & Asami, 1976), is less than normal in endurance trained athletes and increases with the cessation of training (Costill, 1967). Consequently, endurance training undertaken with strength training may reduce the capability of the neuromuscular system to produce maximal power output (Dudley & Fleck, 1987). Therefore, impeding the ability of the neuromuscular system to adapt to the organisation of
efficient motor unit recruitment normally associated with strength training alone (Leveritt et al., 1999).

2.6.2.4. Endocrine Changes

The endocrine system has been identified as playing an important role in regulating growth and development as well as in the acute responses and chronic adaptations to both resistance and endurance exercise training (Chromiak & Mulvaney, 1990). In particular, it has been theorised that the acute changes in serum hormones affect the adaptive response of skeletal muscle to exercise (Antonio, 2000). Two such hormones are testosterone and cortisol, which have been used as markers for measuring anabolism and catabolism responses and adaptations to exercise (Leveritt et al., 1999). Testosterone, a sex hormone is considered a marker of an anabolic state because of its direct and indirect association with increasing protein synthesis (Gurnell, Burrin, & Chatterjee, 2003) via the interaction with other hormones (e.g. growth hormone and IGF-1) and increased intramuscular amino acid uptake and hence ability to stimulate growth (Florini, 1970; Hackney, 1989). Furthermore, testosterone also provides defences against catabolism via the reduction of glucocorticoid receptor binding and the inhibition of muscle glycogen breakdown (Loebel & Kraemer, 1998). In contrast, cortisol, a glucocorticoid released from the adrenal cortex in response to adrenocorticotropic hormone release from the anterior pituitary gland, is considered a marker for a catabolic state because of its function as an inhibitor of amino acid uptake and protein synthesis in peripheral tissues such as muscle (Chattoraj & Watts, 1986; Gurnell et al., 2003; Kaplan, 2000). In addition, it is also associated with alterations in carbohydrate metabolism via promotion of gluconeogenesis as well as mobilisation of triglycerides by lipolysis in response to low glycogen levels (Chattoraj & Watts, 1986; Galbo, 1992; Kraemer, 2000b).

Whilst there is a large degree of variability in the literature concerning the hormonal responses to strength and endurance training, strength training typically evokes an increase in testosterone concentration both during and immediately post-exercise that gradually declines throughout the duration of the recovery period (Gotshalk et al., 1997; Häkkinen et al., 1988; Kraemer et al., 1990; Weiss, Cureton, & Thompson, 1983). In contrast, endurance training typically evokes large increases in cortisol concentration post-exercise which, like the testosterone response following strength training, gradually declines over the duration of the recovery period (Fellmann et al., 1989; Kindermann et al., 1982; MacConi...
Lampman, Schork, & Beitins, 1986; Ronsen et al., 2001; Tabata, Atomi, Mutoh, & Miyashita, 1990; Viru, Karelson, & Smirnova, 1992). However, both strength and endurance training have also been found to increase cortisol (Guezennec, Leger, Lhoste, Aymonod, & Pesquies, 1986; Kraemer, 1988; Kraemer et al., 1993; Kraemer et al., 1998) and testosterone concentrations (Semple, Thomson, & Beastall, 1985; Urhaausen & Kindermann, 1987) post-exercise to varying degrees, respectively.

A number of factors related to exercise have been implicated in determining the magnitude of hormonal changes (Ronsen et al., 2001). These include intensity and duration of exercise, substrate availability (Chattoraj & Watts, 1986; Galbo, 1992; Kraemer, 2000b) and psychological stress (Chattoraj & Watts, 1986). However of these factors, intensity and duration of exercise appear to be the most influential (Fahrner & Hackney, 1998; Gotshalk et al., 1997; Hartley et al., 1972; Kindermann et al., 1982; Kraemer et al., 1990; Loebel & Kraemer, 1998; Viru et al., 1992), as they determine the amount of muscle mass involved in the exercise as well as the total amount of work performed during the training session (Gotshalk et al., 1997). In essence, intensity and duration of training determine the number of muscle fibres stimulated, amount of muscle tissue remodelling, and the amount of muscle tissue repair required consequent to the exercise stress (Kraemer, 2000b).

The influence of intensity and duration of training on hormonal responses has been highlighted in studies that have compared various strength training protocols (Gotshalk et al., 1997; Häkkinen & Pakarinen, 1993; Kraemer et al., 1993; Kraemer et al., 1990). In a study by Gotshalk et al (1997), it was found that high-volume protocols (multiple sets) of moderate-high intensity produced greater testosterone responses than low-volume protocols (single set) of high-intensity. Similarly, with endurance exercise it has been suggested that higher cortisol concentrations occur when endurance exercise intensity is greater than 60% of VO2 max and duration of exercise greater than one hour because of a greater physiological stress and reduction of muscle glycogen stores (Banfi, 1998; Viru et al., 1992).

In terms of concurrent training regimes, it has been proposed that the adaptation to strength training is different when combined with endurance training due to the endurance component creating a more catabolic environment that impedes strength development (Bell et al., 2000; Leveritt et al., 1999), a state that is commonly assessed by the calculation of the testosterone:cortisol ratio (Adlercreutz et al., 1986; Banfi, 1998). The same line of reasoning
has been proposed for the impedance of endurance development, whereby the strength training component may modify the hormonal environment normally associated with endurance training (Leveritt et al., 1999). Such differences in the hormonal environment are thought to “influence the cellular changes related to protein synthesis, neurotransmitter synthesis and subsequent muscle fibre adaptations as well as substrate utilisation and endurance capabilities” (Kraemer et al., 1995).

In one of the few studies that has examined endocrine responses to concurrent training (Bell et al., 2000; Craig et al., 1991; Kraemer et al., 1995), Kraemer et al (1995) reported that a concurrent training programme may result in a significant increase in cortisol, as the COMB group in their study recorded a significant increase in cortisol concentration levels compared with the S and E groups over 12 weeks of training. The increased cortisol concentration was thought to have compromised muscle strength, power and size gains in the COMB group compared to the S group. Interestingly, in the same study, concurrent training did not reduce testosterone levels compared to the S group. However, the increased level of cortisol and unchanged level of testosterone alters the testosterone: cortisol ratio in favour of a catabolic state and as such may be the mechanism responsible for inhibiting strength development in concurrent training.

A similar outcome to that reported by Kraemer et al (1995) was also observed in a study by Bell et al (2000), who investigated the effect of concurrent strength and endurance training on strength, endurance, hormonal status and muscle fibre properties in 45 physically active male and female participants over a 12-week period. Four groups, S, E, COMB and control groups completed the study but no changes were observed in testosterone over the 12 weeks, which was in accordance with the finding of Kraemer et al (1995). However, unlike Kraemer et al (1995), Bell et al (2000) reported that whilst there was a greater increase in urinary cortisol concentration for the COMB group, it was only observed in the women and not the men. Again this suggests that the combination of strength and endurance training may lead to an elevated catabolic state compared to performing the same strength or endurance training separately. The results also suggest that there may be a gender factor involved in hormonal responses to concurrent training, an aspect that requires further investigation.

Whilst research concerning hormonal adaptations during concurrent training studies is limited (Bell et al., 2000; Craig et al., 1991; Kraemer et al., 1995), less is known concerning the acute
responses of concurrent training regimes completed on the same day. The importance of the acute response to concurrent training is that the accumulation of the acute responses to a training stimulus is what determines the adaptation response over time (Bompa, 1994; Brooks et al., 2000; Fleck & Kraemer, 1997), as outlined earlier in this review. Consequently, it is worth mentioning the existing evidence to date on acute hormonal responses to multiple daily training.

The evidence from studies that investigated the effect of repeated bouts of the same type of training suggest that hormonal responses may be changed in response to the second bout completed on the same day involving the same muscle groups (Häkkinen et al., 1988; Ronsen et al., 2001). In one such study, Ronsen et al (2001) compared an initial bout of high-intensity endurance exercise with a second bout of high-intensity endurance exercise completed three hours later and reported that there was a pronounced neuroendocrine response to the second bout of exercise compared to the initial bout with a significant increase in cortisol, epinephrine and growth hormone and a decrease in testosterone levels during and/or after the second bout. The investigators attributed the findings to a number of possible explanations including a reduction in muscle glycogen levels, desensitisation of adrenoreceptors in peripheral tissue, and reciprocal immuno-neuroendocrine regulation. Häkkinen et al (1988) completed a similar study but using eight highly trained weight lifters who completed two successive high-intensity strength training sessions in the one day, separated by four hours of rest. Like Ronsen et al (2001) who reported a different response pattern in the second training session compared to the first, Häkkinen (1988) also reported an altered hormonal response not found during the first training session completed earlier in the day, with significant increases in serum testosterone concentrations during the second training session followed by significant decreases in testosterone after the termination of the training session. A similar pattern was also observed for cortisol.

The implications of the findings by Ronsen et al (2001) and Häkkinen et al (1988) for concurrent training regimes is that athletes may experience a relatively higher stress response during a second bout of exercise using the same muscle groups which may alter the endocrine response during and post-exercise. Consequently, the choice of the length of the recovery period between exercise bouts may greatly affect the physiological responses experienced during and post the second exercise bout. Thus the question then is in what order should the concurrent training sessions be completed? If the endocrine response is determined by the
level of stress encountered during training then the session that comes second may override or impede the response of the earlier session or augment the earlier sessions response. A question that is yet to be answered given that only a few studies have investigated endocrine changes (Bell et al., 2000; Kraemer et al., 1995) and both have used different training regimes for their concurrent trained groups. Subjects used in Kraemer et al (1995) completed endurance training before strength training with 5-6 hours recovery between sessions four days per week, whilst in Bell et al (2000) they completed strength and endurance training sessions on alternate days, six days per week.

It is worth noting that the trainability of the individual has also been implicated as a factor determining the endocrine response to strength training, as changes in serum testosterone levels in Olympic weightlifters have been positively related to changes in strength (Häkkinen et al., 1988). In contrast, endurance-trained male athletes have been reported to have lower total testosterone levels compared to untrained males (Hackney, Sinning, & Bruot, 1988; Wheeler, Wall, Belcastro, & Cummiong, 1984). Further, large inter-individual variability has been found with respect to both trained and untrained subjects in response to exercise, with some subjects showing increases in hormone concentrations whilst others decreases for the same protocol (Viru et al., 1992). Consequently, the more prominent type of training that an athlete undertakes or the type of training that the athlete has the greatest training age in, might ultimately determine the level of endocrine response post-training during a concurrent training regime. Interestingly, in a study that compared changes in testosterone concentrations after strength and endurance exercise of the same duration and perceived exertion in well-trained males experienced in both forms of training, it was found that the response pattern and time course of the changes were similar (Jensen et al., 1991).

Whilst the evidence collected to date regarding hormonal changes in concurrent training regimes and multiple daily training sessions suggest alterations in the relationship between testosterone and/or cortisol responses and strength and endurance development, further research is warranted to examine this relationship.
2.6.3. Acute Mechanisms

2.6.3.1. Residual Fatigue

The acute hypothesis outlined by Craig et al (1991), contends that residual fatigue from the endurance component of concurrent training compromises the ability of the muscle to develop tension during the strength element of concurrent training. This hypothesis was proposed by Craig et al (1991) after observing how different modes of training influenced selected physiological parameters, that upper body development was unaffected by performing running prior to strength training whilst lower body development was impeded. The investigators reported the S group had significantly greater increases in thigh diameter and leg press strength than the COMB group after ten weeks of training when the COMB group performed running immediately prior to strength training. The finding suggests that the sequence and/or schedule of training (i.e. duration between sessions) may have been responsible for the impedance of strength development and not simply the fact of performing strength and endurance training concurrently.

The significance of the proposed mechanism of residual fatigue is that, the level of muscle tension, a critical factor in optimal strength development (Atha, 1981), may be compromised if strength training is completed after endurance training. Therefore, if a reduced level of tension is continually developed during strength training after endurance training, then it is conceivable that a less than optimal level of strength development would take place. Thereby reducing the level of strength adaptation over time compared to when strength training is performed alone. Evidence to support this line of reasoning was outlined earlier in the review under the heading “Effect of Prior Endurance Training on the Acute Strength Response”, with a number of studies reporting that maximal isokinetic (Abernethy, 1993; Bentley et al., 1998; Forsberg et al., 1979; Sherman et al., 1984) and isometric force (Sahlin & Seger, 1995) were reduced in the hours to days following endurance exercise.

The reduction in level of tension generated by the muscles immediately post-training has been attributed to muscular fatigue resulting from an accumulation of metabolites including lactic acid, hydrogen ion and inorganic phosphate (Allen, Westerblad, Lee, & Lännergren, 1992; Maclaren, Gibson, Parry-Billings, & Edwards, 1989; Sahlin, 1992), the level of accumulation increasing with exercise intensity (Baker et al., 1993). The accumulation of these metabolites
causes change at the level of excitation-contraction coupling by reducing sensitivity of the myofilaments to calcium by reducing the number of binding sites available on troponin via competition from other ions (e.g. hydrogen ions) and/or reducing the maximum calcium activated force (Allen et al., 1992; Bigland-Ritchie & Woods, 1984). In addition, the excessive accumulation of hydrogen ions causes a drop in the level of intramuscular pH, which may limit muscular contraction due to a number of rate limiting enzymes being pH sensitive (Pyne, 1994).

Even though there is evidence to suggest that muscular fatigue may not be directly associated with the accumulation of metabolites like lactic acid (Webster, Webster, Crawford, & Gladden, 1993), it is unlikely to be a factor in concurrent training regimes where there are hours of rest between training sessions because metabolites like lactic acid are dispersed shortly after the cessation of exercise (Baker et al., 1993; Bangsbo, Graham, Johansen, & Saltin, 1994) via either oxidation or resynthesis to glycogen in the liver and muscle (Bahr, 1992; Bangsbo et al., 1994). However, the accumulation of metabolites may play a part in concurrent training regimes where strength training immediately precedes endurance training, similar to the protocols used in the studies by Collins and Snow (1993), Dolezal and Potteiger (1998); Gravelle and Blessing (2000) and Nelson et al (1990). Lactic acidosis is generally not a limiting factor in low to moderate intensity continuous exercise because of the low levels of lactic acid accumulated (Brisswalter, Hausswirth, Smith, Vercruyssen, & Vallier, 2000; Galloway & Maughan, 1997; Guezennec, Vallier, Bigard, & Durey, 1996; Le Gallais et al., 1999) and therefore, may not be a limiting factor in a strength training session following an endurance training session. However, if the preceding endurance training session involves periods of high-intensity exercise such as that used by Leveritt and Abernethy (1999), which can increase the level of lactate accumulation, then muscle acidosis may influence the ability of the muscles to generate force in the following strength training session.

In association with the accumulation of metabolites during exercise influencing the level of fatigue post-exercise, it has also been found that fatigue during exercise is related to the predominant type of muscle fibre in the exercising muscle (Kanehisa, Ikegawa, & Fukunaga, 1997). In a study comparing isokinetic forces produced by single as well as repetitive maximal knee extension exercises for resistance-trained, endurance-trained and untrained subjects, it was found that those subjects with a higher percentage of fast twitch fibres in the vastus lateralis were more susceptible to fatigue than those with a high percentage of slow
twitch fibres (Kanehisa et al., 1997). The difference between the subjects was attributed to the endurance-trained subjects supplying a greater part of their energy from aerobic energy sources. This finding was consistent with an earlier finding by Ivy, Sherman, Miller, Maxwell and Costill (1982) during 45s of repeated maximal isokinetic knee extension-flexion contractions. They observed that a person with a higher muscle tissue capacity for aerobic metabolism showed a lower rate of fatigue development. Other studies have also shown a similar relationship (Colliander, Dudley, & Tesch, 1988; Kroll, Clarkson, Kamen, & Lambert, 1980; Suter, Herzog, Sokolosky, Wiley, & Macintosh, 1993; Tesch, Sjödin, Thorstensson, & Karlsson, 1978; Thorstensson & Karlsson, 1976).

Furthermore, in a study comparing the effects of passive and partially active recovery on lactate removal after exhaustive cycle ergometer exercise in endurance- and sprint-trained athletes, it was found that the endurance-trained athletes recovered faster than the sprint-trained athletes (Taoutaou et al., 1996). This finding was attributed to the enhanced ability to remove blood lactate induced by endurance training adaptations such as increased muscle blood flow, which enhances lactate exchange from active muscles to removal sites. The findings by Taoutaou et al (1996) were consistent with the findings of an earlier study by Colliander et al (1988) who also reported that those with a greater percentage of fast twitch fibres showed a slower restoration of force during recovery between strength training bouts than those with a greater percentage of slow twitch fibres.

The findings of the abovementioned investigations suggest that like the endocrine responses to training, the more prominent muscle fibre type an athlete has together with the type of training that an athlete undertakes, and the greatest training adaptations, may be important factors in minimising the effects of fatigue post-training during a concurrent training regime. Whilst the accumulation of metabolites may impair muscle performance immediately following exercise, neuromuscular fatigue, muscle damage and reduced muscle glycogen levels are thought to be the major acute sources of residual fatigue in concurrent training regimes.

2.6.3.2. Neuromuscular Fatigue

Muscular fatigue can stem from a failure at any link in the command chain for voluntary muscular activity that starts in the brain and finishes with the formation of actin-myosin cross
bridges within the muscle (Maclaren et al., 1989). Consequently, muscular fatigue can incorporate both central and peripheral factors. Central factors are those processes that control the discharge rate of motoneurons or more simply the failure to activate the muscle voluntarily (Gandevia, 1992, 2001), whilst peripheral factors refer to those processes distal to the neuromuscular junction (Gandevia, 1992, 2001). Central fatigue sources include malfunction of nerve cells, inhibition of voluntary effort as well as altered input from muscle receptors, for example muscle spindles and tendon organ (Gandevia, 2001; Maclaren et al., 1989). On the other hand, peripheral fatigue sites include the neuromuscular junction, the calcium release mechanism (i.e. activation) and the sliding filaments (contractile processes) (Maclaren et al., 1989). It has become generally accepted that peripheral fatigue results in a progressive increase in neural input to the active skeletal muscles in order to initiate the recruitment of additional muscle fibres in order to offset any decrement in force production by fatiguing muscle fibres (St Clair Gibson, Schabort, & Noakes, 2001). Whilst muscle fatigue can be measured in terms of reduced force generating capacity, it is also accompanied by many other measurable changes such as changes in muscle activation, slowing of muscle conduction velocity and muscle contraction speed (Bigland-Ritchie & Woods, 1984). In an attempt to determine the source of fatigue, that is whether it was due to central or peripheral factors, studies have been completed using a variety of investigative techniques including EMG, electrical stimulation (ES) and twitch interpolation (TW).

Central Fatigue

EMG – a decline in the EMG with a corresponding reduction in force (Green, 1995).

ES and TW – an increase in force above that achieved during a maximal voluntary contraction MVC following the application of a superimposed electrical stimulation (Allen, Gandevia, & McKenzie, 1995b; Green, 1995).

Peripheral Fatigue

EMG – an increase in EMG whilst force is maintained or decreases (Basmajian & De Luca, 1985).
ES and TW – a reduction in both the artificially induced force from the superimposed electrical stimulation and that achieved via a MVC (Bigland-Ritchie, Furbush, & Woods, 1986).

The majority of research in the area of neuromuscular fatigue using the above techniques has been centred on fatigue during and following static isometric contractions (Babault, Pousson, Ballay, & van Hoecke, 2001; Häkkinen & Komi, 1986). However, some research has investigated the effects of endurance exercise on neuromuscular fatigue (Bentley, 1998; Lepers et al., 2000; Paavolainen, Nummela, Rusko, & Häkkinen, 1999; Sargeant & Dolan, 1987; St Clair Gibson et al., 2001).

In order to determine the level of muscle activation of the quadriceps muscle following 30 minutes of cycling exercise at the D\textsubscript{max} lactate threshold, with four times 60 second efforts at 120% of the workload at VO\textsubscript{2} max, Bentley (1998) used maximal force generating capacity and EMG in conjunction with an electrical stimulation technique. The results indicated that there was a significant reduction in maximal voluntary isometric force and iEMG of the rectus femoris and vastus medialis and lateralis muscles ten minutes post-exercise that remained for a period of six hours. A significant increase in the activation deficit of the quadriceps muscle was also found at both the ten-minute and six hour post-exercise time points. The reduction in muscular strength was attributed to a reduction in the level of activation of the quadriceps from both the central nervous system as well as a disruption of the muscle fibres thereby inhibiting muscle contraction.

Using a combination of techniques, similar to those used by Bentley (1998), Lepers and colleagues (2000) investigated the effects of two hours of continuous cycling at a power output corresponding to 65% of VO\textsubscript{2} max in eight well-trained male cyclists and triathletes on contractile and neural properties of the quadriceps muscle. They found that muscular peak torque was reduced by 11-15% for concentric, eccentric and isometric contractions immediately post-exercise and was associated with a reduction in the maximal EMG activity of the vastus medialis and lateralis muscles. Further, a significant reduction in maximal twitch tension was also found. The authors attributed the changes to peripheral mechanisms that included alterations of the surface action potential or M-wave (increased duration of the action potential and a reduction in the peak-to-peak amplitude and total area of the action potential) and failure in excitation-contraction coupling. They interpreted the increased action
potential duration in the vastus medialis and lateralis as a slowing of conduction velocity along the membranes of muscle fibres and the reduction in peak-to-peak amplitude and total area of the action potential to presynaptic and/or endplate fatigue.

Neuromuscular fatigue similar to that outlined above has also been found following endurance bouts of running (Paavolainen et al., 1999) and downhill walking (Sargeant & Dolan, 1987). These results confirm that neuromuscular fatigue from an endurance training session could influence another training session completed within close proximity to the finish of the session. However, unlike the effects of endurance training on the recovery of force generating capacity, no research has been completed to date on the effect of prior strength training on the completion of endurance exercise. The evidence indicating that maximal force generating capacity and neural activation of muscles following strength training sessions are reduced suggests that endurance performance following strength training sessions could also be affected (e.g. reduced time to fatigue, increased metabolic cost) (Behm, Reardon, Fitzgerald, & Drinkwater, 2002; Häkkinen, 1990; 1992; 1993; Kroon & Naeije, 1991; Linnamo et al., 1998).

Häkkinen (1993) investigated neuromuscular fatigue and recovery of force generating capacity in ten male and nine female strength-trained athletes following heavy resistance exercise consisting of 20 repetitions of their squat 1 RM in one-training session. Maximal voluntary neural activation (measured via iEMG) and bilateral isometric force were measured pre, immediately post, at one hour, two hours and one and two days post-exercise. There was a reduction in strength of approximately 24 and 20% respectively in males and females immediately post-exercise and a significant decrease in maximal iEMG. Both parameters improved over the days following exercise but were still below pre-training levels two days post-training. This finding suggests that high-intensity resistance training may result in “considerable acute fatigue in the neuromuscular system leading not only to the decreased force production capacity of the muscles but also to a decrease in the voluntary neural activation of the exercised muscles” (Häkkinen, 1993). This study supported a previous finding by the same investigator (Häkkinen, 1992) that showed that the completion of two successive strength training sessions in one day produced a greater decrease in maximal strength accompanied by a shift (worsening) in the force time curve for the second training session. Further, a reduction in iEMG was also observed during the second session. The investigator suggested that the results for the second session indicated that acute fatigue might...
not only occur in the contractile components of the examined muscles but also in the central nervous system as well.

The studies by Häkkinen (1990; 1992; 1993) and others (Kroon & Naeije, 1991; Linnamo et al., 1998) all used multiple training sets and showed large reductions in force generating capacity and changes in neuromuscular parameters post-exercise. The same has also been found in the comparison of single sets of strength training (Behm, Reardon et al., 2002). For example, Behm et al (2002) compared the effects of single sets of 5, 10 and 20 RM elbow flexion in 14 subjects on the recovery of maximum isometric force, EMG and muscle activation (twitch interpolation) at 30 seconds, one, two and three minutes post-exercise. Even though no significant difference was found between these variables in the three minutes of measurement post-exercise, it was found that as the RM became greater so too did the detrimental effects of the exercise. Furthermore, the recovery period required to restore full function was found to be greater than the three minutes currently widely recommended to used between training sets (Fleck & Kraemer, 1997).

The neuromuscular fatigue following strength training may be related to the type of muscle contraction involved in the exercise for it has been found that exercise involving eccentric contractions produce greater decrements in neuromuscular performance than exercise with only concentric contractions (Ebbeling & Clarkson, 1989; Komi & Viitasalo, 1977; Kroon & Naeije, 1991; Sargeant & Dolan, 1987). For example, Komi and Viitasalo (1977) found that following eccentric exercise on an electromechanical dynamometer, there was a decrease in maximal strength as measured by isometric knee extension and an increase in neural activity at a given muscle tension both immediately and two days post-exercise. Similarly, Kroon and Naeije (1991) reported that compared to concentric and isometric exercise which had smaller and short responses, eccentric exercise evoked greater and longer lasting responses post-training as measured by changes in EMG amplitude and mean power frequency. The decrements in neuromuscular performance following eccentric exercise are thought to be due to the mechanical stress during the eccentric work component of the muscle contraction causing structural damage to the initially recruited motor units, necessitating the recruitment of additional motor units to maintain force production (Ebbeling & Clarkson, 1989).
central and peripheral sources of fatigue as a result of a reduction in neural input and/or changes in contractile properties of the muscle. Therefore, it is possible that regardless of the sequence of concurrent training, some form of residual fatigue from the proceeding training activity may reduce the quality of a subsequent training activity and thus lead to a less than normal training induced response associated with the second training activity (Leveritt et al., 1999). However, as yet the influence of the sequence of concurrent training on neuromuscular fatigue has not been investigated.

The above discussion outlines the effects of residual fatigue from the perspective of neuromuscular fatigue. However, two other sources have been proposed as contributing to the residual fatigue mechanism - muscle damage and the depletion of muscle glycogen stores (Leveritt et al., 1999). Whilst both of these sources of residual fatigue also contribute to neuromuscular fatigue they have been separated to enable the characteristics specific to each source to be addressed.

2.6.3.3. Muscle Damage

Exercise-induced muscle damage has been reported to result in a number of local and systematic changes including disruptions to the sarcolemma, Z discs, myofibres and excitation-contraction coupling processes, swollen mitochondria (Armstrong et al., 1983; Behm, Baker, Kelland, & Lomond, 2001; Fridén, Sjöström, & Ekblom, 1983; Stauber, 1989) and an elevation of muscle proteins in the blood (Behm et al., 2001; Newham, Jones, & Clarkson, 1987; Nosaka & Clarkson, 1992) as well as force production decrements (Behm et al., 2001; Evans & Cannon, 1991; Kuipers, 1994; Newham et al., 1987) and neuromuscular deficits (muscle inactivation) (Behm et al., 2001). The exact stimulus for muscle hypertrophy and strength gains following resistance training are still unclear. However one possible explanation may be that the high mechanical stress caused by high levels of eccentric work and the associated damage and repair process may initiate the compensatory process for hypertrophy (Folland, Chong, Copeman, & Jones, 2001) via an increased rate of muscle protein turnover (Chesley et al., 1992; Fielding et al., 1991). Whilst Folland et al (2001) provided evidence to suggest that this may not be the case due to finding that an acute bout of eccentric muscle damage did not accentuate training induced strength gains, their findings do pose the question as to what effect does the muscle damage incurred during one bout of exercise have on subsequent exercise bouts.
To date, the degree of muscle damage following bouts of strength and/or endurance training in concurrent training studies has not been investigated, using either direct (ultrastructure damage via electron microscopy) or indirect methods (creatine kinase, a marker of the magnitude of muscle damage) (Hortobágyi & Denahan, 1989; Nosaka & Clarkson, 1992). However, there is evidence to suggest that muscle damage may be a possible source of residual fatigue during concurrent training (Leveritt et al., 1999). The majority of studies (Craig et al., 1991; Dolezal & Potteiger, 1998; Hortobágyi et al., 1991) that have utilised running as the endurance training modality have reported interference in the development of strength, particularly when isotonic strength training has also been utilised, whilst the majority of studies that have utilised cycling (McCarthy et al., 1995; Sale et al., 1990a) or arm crank training (Abernethy & Quigley, 1993) as the endurance training modality have reported no interference (Leveritt et al., 1999). These findings suggest that the modality of endurance training coupled with the type of resistance training used may be a factor that influences the level of strength and endurance adaptation. A possible explanation for this association may be the type of muscle contractions that are incorporated in the two types of training. Running involves both concentric and eccentric contractions whilst cycling and arm cranking are essential concentric only. Hence, those studies that incorporated running with isotonic strength training may have increased the level of muscle damage experienced by the muscle groups involved.

Support for this line of reasoning has been found in studies that have investigated the extent of muscle damage following various types of exercise (Armstrong et al., 1983; Clarkson et al., 1985; Jamurtas et al., 2000; Newham, Jones, & Edwards, 1983). It is widely documented that the type of exercise undertaken is a critical factor in influencing the degree of muscle damage incurred following exercise, with eccentric contractions producing a greater amount of muscle damage and larger elevations in creatine kinase in the blood than concentric exercises (Armstrong et al., 1983; Clarkson et al., 1985; Enoka, 1994; Hortobágyi & Denahan, 1989; Jamurtas et al., 2000; Newham, McPhail et al., 1983). Consequently, the increased level of muscle stress that is reported to occur with running may interfere with strength and/or endurance development due to a greater degree of muscle damage compared with cycling (Noakes, 1991; Spitler, Alexander, Hoffler, Doerr, & Buchanan, 1984).
Whilst it is possible that endurance exercise involving relatively large amounts of eccentric muscle activity may impair performance in a subsequent strength training session, this source of fatigue has been considered unlikely to affect long term impairment of strength development by some investigators (Leveritt et al., 1999) for two reasons. First, because muscle damage after repeated bouts of either strength or endurance exercise, termed the ‘repeated bout effect’ (Hortobágyi & Denahan, 1989), is reported to be minimal or induces no damage compared with the initial exercise bout (Ebbeling & Clarkson, 1989; Evans et al., 1986; McHugh, 1999; Newham et al., 1987). Second, the level of damage after strength training would be greater than that induced after endurance training and as such S groups would experience impaired strength development similar to the COMB group yet this is not a common occurrence (Leveritt et al., 1999).

An example of the effect of repeated bouts of strength training was provided in a study by Newham et al (1987) who examined plasma CK levels following three bouts of training over a four-week period. They found that after the first bout of exercise, where there were very high plasma CK levels (1,500-11,000 U.L\(^{-1}\)), the second and third bouts, two and four weeks later, showed no significant effect on plasma CK compared to pre-training CK levels. Further support for the repeated bout effect comes from investigations that have used untrained subjects performing high-intensity training to assess the impact on muscle damage via the CK response and have reported large increases in the range of 300-11,000 U.L\(^{-1}\) from 24 to 72 hours following one training session (Dolezal et al., 2000; Jamurtas et al., 2000; Newham et al., 1987; Tiidus & Ianuzzo, 1983). In contrast, studies using trained subjects have reported lower post-training CK levels (109-800 U.L\(^{-1}\)) (Dolezal et al., 2000; Robinson, Williams, Worthington, & Carter, 1982). It has been proposed that the reduced CK levels following repeated bouts of exercise are the result of connective tissue undergoing structural changes due to forced lengthening making muscle fibres resistant to further damage (Appell, Soares, & Duarte, 1992; Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1989; Kuipers, 1994).

In a study of triathlon (Guezennec et al., 1996), the effects of a prior bout of cycling on running performance compared to an isolated bout of running in trained athletes were examined. It was found that the prior exercise, which did not include eccentric contractions, caused an elevation in plasma CK post-training. The investigators attributed the difference to muscle fibres that were not generally recruited during an isolated running event being used in the triathlon run and becoming damaged because they were unaccustomed to the stress placed
on them. Whilst this is an isolated study it does highlight that further research is required to explain the impact that prior exercise has on muscle damage and the effect on subsequent endurance activities. Furthermore, more research focusing on the possible interaction between the modality of strength and endurance training and strength and endurance development needs to be completed.

2.6.3.4. Glycogen Depletion

The depletion of muscle glycogen has been proposed as a contributing factor to muscle fatigue (Costill & Hargreaves, 1992) because of the well documented evidence that indicates that an acute bout of endurance exercise can considerably reduce muscle glycogen levels (Costill, Bowers, Branam, & Sparks, 1971; Costill, Sparks, Gregor, & Turner, 1971; Gollnick, Armstrong, Saubert et al., 1973; Saltin & Hermansen, 1967; Sherman et al., 1984; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990). Consequently, the performance of exercise using the same muscle groups in the period of time before muscle glycogen levels have been replenished after endurance exercise would be restricted, either in the level of force generation (exercise-intensity) or time duration of the exercise (Grisdale, Jacobs, & Cafarelli, 1990). Therefore, it is plausible that strength training performance following endurance exercise would be impaired. However, this line of reasoning has been questioned because of the findings of a number of concurrent training studies (Dudley & Djamil, 1985; Hickson, 1980) and individual endurance studies (Sahlin & Seger, 1995; Sherman et al., 1984). As was outlined earlier, the level of training undertaken by the subjects in the study by Dudley and Djamil (1985) was considerably less than that undertaken by the subjects in the study by Hickson (1980) yet both experienced impeded strength development. Further, the subjects in the study by Hickson (1980) experienced continued endurance development over the weeks that strength training declined even though they trained both the strength and endurance training sessions on the same day as opposed to Dudley and Djamil (1985) subjects who trained on separate days. Whilst this suggests that muscle glycogen depletion was not a factor in the impeded strength development, the findings may simply reflect an insufficient volume or intensity of training to significantly reduce muscle glycogen stores to a level that would impede endurance development but was enough to influence strength development. Support for this suggestion can be found in those studies that reported impeded strength development but not aerobic capacity (Bell et al., 2000; Craig et al., 1991; Dolezal & Potteiger, 1998; Dudley & Djamil, 1985; Hortobágyi et al., 1991), with the
majority of these studies utilising short duration (less than 40 minutes) endurance training sessions of moderate to high intensity exercise where significant muscle glycogen depletion is unlikely due to the short duration of exercise.

Further evidence that the volume and intensity of training in concurrent training studies may have been insufficient to significantly reduce muscle glycogen stores to a level that would impede endurance development but sufficient to influence strength development can be found in the study by Costill et al (1971) who investigated muscle glycogen utilisation during prolonged exercise on successive days. Their subjects ran 16.1km on three successive days on a treadmill at 80% of VO2 max. Muscle biopsies were taken from the vastus lateralis before and after each training session and revealed that whilst muscle glycogen depletion was greatest after the first session, successive days of training also produced marked reductions in muscle glycogen depletion. The results showed that following a high level of depletion muscle glycogen levels were not restored to pre-exercise levels before the second training session commenced the following day. Further, the third day’s training session commenced at a lower level than the previous day’s exercise bout. These findings suggest that, even though subjects showed notable reductions in muscle glycogen levels, their endurance performances were not impeded. In addition, in a later study (Costill et al., 1988) that investigated the effects of ten days of intense repeated swimming exercise on muscle glycogen content and swimming performance, the investigators reported that whilst muscle glycogen levels were reduced following the ten days of exercise, there was no reduction in swimming performance even though subjects did experience difficulty in completing the training sessions.

Sherman et al (1984), as outlined earlier, attributed the decline in isokinetic leg-extension maximal peak torque following a marathon to muscle glycogen depletion. However, over the days following the marathon when muscle glycogen levels had returned to normal, maximal peak torque was still reduced compared to pre-marathon values. This finding was supported by Sahlin and Seger (1995) but suggested that the initial recovery was due to muscle energetics or electrolyte balance but the ongoing force reduction was due to damage of structural elements of the muscles involved or to non-metabolic factors.

Whilst there is evidence to support the concept that the ongoing force reduction following exercise is due to damage of structural elements, the interaction between muscle damage and glycogen depletion may also be a contributing factor. In an examination of the glycogen
repletion of muscle fibres following 45 minutes of eccentric exercise on a cycle ergometer (O'Reilly et al., 1987), it was found that there was delayed repair of ultrastructure damage along with impaired repletion of muscle glycogen. Whilst the relationship between delayed glycogen repletion and myofibrillar damage is unclear, it is possible that alterations in the membranes of muscles involved in the exercise may interfere with glucose uptake by the cells (O'Reilly et al., 1987).

The above literature review outlines the effects of prior endurance activity on force generating capacity and muscle glycogen post-exercise. Previous research has also shown that muscle glycogen levels can also be reduced following strength training exercise (Pascoe, Costill, Fink, Robergs, & Zachwieja, 1993; Robergs et al., 1991; Tesch, Colliander, & Kaiser, 1986). In a study by Tesch, Colliander and Kaiser (1986) who investigated the effects of intense, heavy-resistance training on muscle metabolism, it was found that muscle glycogen stores were reduced by as much as 25% post-exercise. The training consisted of five sets of front and back squats, leg-extensions and leg-press with 6-12 repetitions. Using similar protocols, Pascoe et al (1993) and Robergs et al (1991) also reported reduced muscle glycogen levels. Taken together these findings highlight that depending on the intensity and duration of the strength training protocol, muscle glycogen can be reduced. Whether there is potential for reduced muscle glycogen levels to interfere with endurance performance if completed within close proximity to the endurance training session, is unknown in light of the findings by Costill and colleagues (Costill, Bowers et al., 1971; Costill et al., 1988).

It is worth noting that there is evidence that muscle glycogen depletion is not uniform across muscle fibre types but differs between fibre types with the level of exercise intensity (Gollnick, Armstrong, Sembrowich et al., 1973; Gollnick, Piehl, & Saltin, 1974; Pascoe & Gladden, 1996). Muscle glycogen is depleted from type I fibres first during low-moderate intensity exercise followed by type II fibres if exercise is continued (Maclaren et al., 1989). During extended high-intensity exercise, type II fibres are progressively recruited as the glycogen levels of type I fibres are reduced (Gollnick, Armstrong, Sembrowich et al., 1973). Consequently, it is possible for an athlete to exercise to fatigue because of glycogen depletion from specific muscle fibres, while glycogen remains in adjacent fibres within the muscle (Brooks et al., 2000). This concept of selective depletion of muscle fibres is important because in studies that examined the relationship between muscle glycogen and quadriceps strength (Jacobs, 1981; Jacobs, Kaiser, & Tesch, 1981), it has been found that muscle strength
was only reduced when the glycogen content of the muscle was below 50 mmol.kg$^{-1}$ and was most reduced in subjects who had a predominant number of fast twitch fibres (>50%). This might suggest that the type of athlete (endurance or strength orientated) and subsequent training status of the athlete might also be factors that determine the level of recovery of muscle glycogen stores post-exercise and hence force generating capacity. In addition, diet may also be an intervening factor both during exercise and in the recovery of glycogen stores post-exercise.

Previous research has shown that the rate of muscle glycogen resynthesis after depletion is dependent on the type of diet (Costill, Bowers et al., 1971; Hultman & Bergstrom, 1967; Ivy, 1991) and the timing of food ingestion post-exercise (Ivy, Katz, Cutler, Sherman, & Coyle, 1988; Tsintzas & Williams, 1998). Glycogen synthesis occurs in biphasic fashion with a rapid phase of resynthesis to near pre-exercise levels within 24 hours whilst the gradual increase in muscle glycogen stores to above-normal levels over subsequent days represents the slow phase (Friedman, Neufer, & Dohm, 1991; Ivy, 1991; Pascoe & Gladden, 1996). The rate of restoration of muscle glycogen during the first 24 hours is dependent upon the amount of carbohydrates present in the diet, with a carbohydrate-rich diet resulting in greater replenishment (Costill, Bowers et al., 1971; Ivy, 1991). In addition, the ingestion of carbohydrates shortly after the cessation of exercise has been shown to increase the rate of restoration of muscle glycogen compared to if the carbohydrate source is delayed for an hour or more (Ivy et al., 1988). In light of the above, diet including the timing of ingestion both during and after exercise, may have implications for concurrent training sessions by determining the extent of muscle glycogen depletion during exercise and the speed and extent of recovery between training sessions, a point worthy of further investigation.

Apart from reduced muscle glycogen levels being associated with fatigue during and post-exercise, low muscle glycogen levels following prolonged exercise have also been found to cause transient increases in the transcription of a number of metabolic genes in both rodents and humans including muscle glucose transporter GLUT-4 (MacLean, Zheng, & Dohm, 2000; Neufer & Dohm, 1993), hexokinase II (O’Doherty, Bracy, Osawa, Wasserman, & Granner, 1993), glycogen synthase, pyruvate dehydrogenase kinase 4, uncoupling protein 3 and lipoprotein lipase during the hours following exercise (Pilegaard et al., 2002). The significance of these responses is that they reflect the metabolic recovery dynamics in skeletal muscle post-exercise. Furthermore, it has been suggested (Pilegaard et al., 2000; Williams &
Neufer, 1996) that the metabolic adaptations that take place in skeletal muscle due to exercise may be the result of the cumulative effects of transient increases in transcription during the recovery period following each exercise bout (Pilegaard et al., 2002). Because the transcriptional activation of a number of genes occurs within the first few four hours following exercise (Pilegaard et al., 2002), it is possible that this process may be interrupted if concurrent training sessions are completed prior to the response of the initial bout of exercise reaching its peak. This may result in a delay in the restoration of muscle glycogen levels to pre-exercise levels. Whilst there is no evidence to indicate that metabolic genes respond differently to repeat or different types of exercise, as was the case with neuromuscular fatigue and endocrine mechanisms, it is possible that the metabolic responses to concurrent training might be affected similar to the speculated mechanism associated with impedance of muscle hypertrophy, as outlined earlier.

Even though there is conflicting evidence as to the impact that muscle glycogen levels have on force production, it is possible that the depletion of muscle glycogen may be a contributing source to residual fatigue in concurrent training, particularly if performed on the same day or if there is insufficient restoration of glycogen stores following previous exercise bouts. However, specific research addressing the sequence, scheduling of training and volume and intensity of training in addition to the influence of pre and post-training glycogen levels on the responses of metabolic genes is necessary before the role of muscle glycogen depletion on strength and endurance development within concurrent training regimes is known.

2.7. Summary

The body’s adaptation to a training stimulus is the accumulation of the body’s acute responses to single bouts of the stimuli. In the case of strength and endurance training, the magnitude and duration of the acute response is determined by the intensity and duration of the activity and may be mediated by the training status of the individual. Strength training typically evokes increased synthesis of myofibrillar proteins and hence muscle hypertrophy as well as reductions in mitochondrial volume and density and alterations in enzymes associated with aerobic processes. In contrast, endurance training evokes changes in aerobic processes like increased respiratory capacity and enzyme activity resulting in a greater aerobic capacity. The implication of the differences in the responses to strength and endurance training is that, the
completion of the two types of training within the one training regime, commonly termed concurrent training, may mean that the types of training are incompatible.

The available evidence from the studies completed in the area of concurrent training suggests there may be impedance of strength and/or endurance adaptations when the two types of training are trained concurrently. Whilst there are a number of studies that have shown impedance of strength or endurance development following concurrent training regimes, there are also studies that have found no impedance of strength and endurance qualities. In light of these contrary findings, the benefit of training strength and endurance sessions concurrently is inconclusive. However, it does appear though that strength adaptations may be more sensitive to concurrent training regimes than aerobic adaptations. Further the sequence that the concurrent training sessions are completed in as well as the duration of the recovery period between training sessions may be influencing factors.

To date few studies have investigated the source of the so-called impedance of strength and endurance adaptations and as such our understanding of the effects concurrent training on the chronic and acute mechanisms that may be responsible for this incompatibility are unclear. However, a number of mechanisms have been proposed including overtraining, alterations in motor unit recruitment, changes in hormonal responses and muscle fibre hypertrophy along with residual fatigue resulting from neuromuscular fatigue, muscle damage and muscle glycogen depletion. Whilst some of these mechanisms have been questioned (e.g. overtraining) and others are only speculative as they have not been previously investigated (e.g. changes in mRNA of selected genes associated with muscle hypertrophy), the role each mechanism plays in concurrent training responses and hence adaptation, is unknown.
CHAPTER 3

General Methodology & Materials

3.1. Introduction

Three studies were completed as part of this investigation of concurrent training. This chapter outlines the general experimental methodology and materials used during the respective studies. Because of the similarity of the experimental methodology and protocols for Studies 1 and 2, the methodology outlined below applies to both projects unless otherwise indicated. However, because Study 3 was completed at the Copenhagen Muscle Research Centre, Denmark, using different experimental protocols, facilities and equipment, the experimental methodology used in that study is outlined separately at the end of this Chapter. The specific experimental design of Studies 1, 2 and 3 are outlined at the beginning of Chapters 4, 5 and 6, respectively.

STUDIES 1 and 2

3.2. Subject Preparation

All subjects were screened for health status and risk factors using a Pre-Participation Health Status Assessment Questionnaire (Appendix A) prior to participation. Each subject was provided with a detailed description of the purpose of the tests, the testing procedures and the potential risks involved with the study (Study 1 – Appendix B, Study 2 – Appendix C) and was required to sign an informed consent form prior to participation (Appendix E).

The experimental procedure was approved by the Southern Cross University Human Research Ethics Committee (Approval number: ECN-01-37).
Prior to each test/training session, all subjects were required to:

1. Avoid participating in any strenuous exercise within 24 hours of a test/training session.
2. Refrain from consuming any caffeine, supplements or drugs, which may affect their performance, throughout the test/training days.
3. Refrain from consuming any alcohol within 24 hours of a test/training session.
4. Refrain from consuming any food or drink (except water) within two hours of a test/training session.

(Mahler, Froelicher, Houston Miller, & York, 1995)

In addition, to limit the possible impact of prior resistance training on the test results, the subjects were asked to refrain from participating in any leg strength training within 48 hours of each test/training session.

### 3.2.1. Subject Diet Monitoring

Because of the sensitive nature of some of the test variables to food and fluid intake, each subject was required to maintain a dietary intake diary for the control day, from the time they awoke in the morning till after that day’s last test session. The subjects were then required to replicate this dietary intake on each subsequent test/training day for the same time period. The subjects were also asked to keep their dietary intake to what they would normally consume over the course of a day. During Study 2 the subjects were also required to fast for 12 hours before the morning blood specimen for the determination of resting hormone concentrations (Rose, 1995).

### 3.2.2. Familiarisation Session

A familiarisation session was completed in the week prior to commencing testing. During this session the subjects were familiarised with the gas collection equipment, the cycle ergometer used in the VO$_2$ max and efficiency tests, the electrical stimulation equipment and the strength training equipment used in the respective test sessions. The subjects were also fully instructed on all the test procedures at this time.

To ensure that the subjects were familiar and comfortable with the procedures and the equipment to be used in the various tests, a series of sub-maximal trials were completed...
during the familiarisation session. This entailed the subjects undertaking a sub-maximal cycle ergometer test and a number of isometric contractions on the leg-extension testing chair including muscle activation (twitch interpolation) trials.

The sub-maximal cycling ergometer familiarisation required the subjects to wear the headset and mouth piece to be used in the cycling VO₂ max and efficiency tests, to ensure familiarisation with the sensation of breathing through such a device, and followed the same protocol including warm-up, as that used in the VO₂ max test (refer VO₂ max protocol). However, the sessions were stopped when a subject reached 70% of his age predicted maximum heart rate (HR\text{max}), calculated from the following equation.

\[
\text{Age predicted HR}_{\text{max}} = 220 - \text{subject’s age}
\]

The cycle ergometer exercise also served as the warm-up for the muscle activation familiarisation session, which required the subjects to undertake a series of progressively stronger twin shocks to the quadriceps femoris muscle, during and after a maximal voluntary contraction (MVC), until the subjects reached their maximal tolerable current or no further increases in isometric force were recorded (Shield, 2003). The values obtained from the familiarisation session served as a guide to the levels that were to be used in the actual muscle activation testing sessions. The use of maximal stimulation levels enabled subjects to be aware of the sensation associated with the twitch interpolation technique.

The familiarisation session was also used to determine each subject’s leg- and bench-press one-repetition maximum (1 RM) and lat pull-down six-repetition maximum (6 RM). These strength assessments were performed after a period of rest (10 minutes) following the completion of the muscle activation trials.

3.3. Protocols

3.3.1. Muscular Strength Assessment (1 RM and 6 RM)

The leg- and bench-press 1 RM and lat pull-down 6 RM muscular strength tests were used to evaluate each subject’s maximal level of strength in a single dynamic movement, in order to determine the loads (percentage of 1 RM, 6 RM or equivalent amount of work) to be used in
the four strength training sessions in Study 1 (S = strength, SE = strength endurance, H = hill cycling, SUL = upper and lower body strength). The SUL protocol was also used for the weight training sessions in Study 2.

Prior to commencing the test, the subjects warmed up on a cycle ergometer for five-minutes at a workload of 1 watt per kilogram (kg) of gross-body-mass (GBM) and performed light stretches of the major leg muscle groups (quadriceps, hamstrings and calves).

The 1 RM has been defined as the weight that can be successfully lifted no more than once, through a specific range of movement (ROM) (Sale, 1991). Therefore, a standardised ROM was used for all tests. During the leg-press the subjects lowered the weight to a flexed knee angle of 90° (Hoeger, Hopkins, Barette, & Hale, 1990), measured manually using a goniometer. The depth of the movement (distance in metres) was also measured to enable the calculation of the amount of work completed per training session, as the S, SE and H strength training sessions were matched for the amount of work completed. The subjects were positioned in a reclining-seated position in the leg-press machine and the safety stops adjusted to accommodate each individual’s limb length. The seat, foot platform and safety stop positions were recorded for each subject to ensure standardised positioning across the strength training sessions. The subjects were required to maintain a shoulder width foot pattern on the leg-press platform throughout the trials.

The subjects were given two standardised sub-maximal warm-up trials with descending repetitions (10 and 5) with progressively heavier loads (150 and 250% of GBM) before performing a series of one-repetition trials with progressively heavier weights to determine their 1 RM (Humphries et al., 1999; Sale, 1991). The weight was increased in increments between 2.5 and 20 kg until the subject could not complete the lift (Murphy & Wilson, 1997). The heaviest weight lifted across the trials was used for estimating of each subject’s 6 RM, calculated as 85% of each subject’s 1 RM (Feigenbaum & Pollock, 1997; Hoeger et al., 1990), and corresponding 20-repetition load (equal amount of work) for the S and SE training sessions, respectively. The subjects were given three minutes rest between each trial to minimise the development of fatigue (Sale, 1991; Weiss et al., 1983; Willoughby, 1991).

The same procedure, as outlined above for the leg-press, was used for the bench-press and lat pull-down strength assessments except that the load used for the warm-up sets was 60 & 80%
and 50 & 70% of GBM, respectively. The bench-press movement was standardised with the bar being lowered smoothly to touch the chest before being lifted to the starting position and were undertaken in a Smith-machine, which provided mechanical stops and restricted range of motion to a specific line, thereby reducing the skill demand required and offering greater safety (Young & Bilby, 1993). A protocol previously used by other researchers (Young & Bilby, 1993) and recommended by the Australian Institute of Sport for bench-press strength testing (Logan, Fornasiero, Abernethy, & Lynch, 1998). The lat pull-down movement was completed to the front of the head and required the bar to be lowered to the line of the top of the shoulders. A 6 RM protocol was used for the lat pull-down exercise instead of a 1 RM protocol to help reduce the stress on the shoulder complex that would be encountered using a 1 RM protocol from a fully extended shoulder position.

3.3.2. Maximal Voluntary Isometric Contraction Assessment

Prior to each strength training session and cycling efficiency test, the subjects completed an identical series of MVCs (right leg only) at 70° of knee flexion on a custom-built testing chair. Seventy degrees of knee flexion was chosen due to its association with force production during the down stroke of the cycling pedalling action (Cavanagh & Sanderson, 1986). The subject’s right leg was used because it corresponded with their dominant leg (determined as their preferred kicking leg).

The subjects were given a series of progressive warm-up trials before performing the three MVCs (Bennet & Stauber, 1986). The subjects performed the contractions in response to a verbal command and held the contractions for a period of three seconds. The duration of the contraction was standardised by the use of an auditory signal (buzzer), which was triggered by the onset of force generation (5-25N) and again three seconds after the first signal (Shield, 2003). In addition, a standardized verbal encouragement phrase was used throughout all MVC trials to ensure maximal effort. The highest torque value across the three maximal trials was recorded as peak torque whilst the average of the highest torque value in each maximal trial was recorded as mean torque. The subjects were given 90 seconds rest between trials (Murray, Baldwin, Gardner, Sepic, & Downs, 1977).
3.3.3. Muscle Activation (twitch interpolation)

During the MVC session each subject was also assessed for voluntary and involuntary muscle activation using a twitch interpolation technique, a technique widely accepted in the assessment of muscle activation (Allen et al., 1995b; Behm, St-Pierre, & Perez, 1996; Belanger & McComas, 1981; Gandevia, 2001). This technique involved applying increasing levels of electric shocks to the quadriceps femoris muscle and observing whether force levels increased. By applying an electrical stimulus during a voluntary contraction and immediately after when the muscle was relaxed, the level of muscle activation was determined. This method was used for the determination of whether a peripheral or central fatigue component contributed to the isometric strength results in the three hours post-strength training (Allen et al., 1995b; Gandevia, 2001; Shield, 2003).

Three twitch interpolation trials were carried out in addition to the MVC trials outlined above. A supra-maximal twin electrical stimulus (50-100 ms in duration) was imposed during a voluntary maximal contraction and five seconds after the first stimulus, when the muscle has relaxed. The level of supra-maximal stimulation was determined during the familiarisation (warm-up) trails, by applying in one minute intervals, progressively stronger and stronger twin shocks to the muscle until the subjects reached their maximal tolerable current or no further increases in contraction force was noted. The level of stimulation was then increased by 10% to provide a supra-maximal level. The first (superimposed) stimulus was triggered by a reduction in force (determined over a 200 ms period) to a level that represented 95% of each subjects’ pre-recorded MVC force. A delay of 300 ms occurred between the registering of a reduction in MVC force and the administering of the stimulus. The difference between the force value obtained immediately prior to the first stimulation and the peak force value obtained in response to the stimulation was calculated as the superimposed response. The second stimulus administered five seconds after the first and during muscle relaxation, was used as a control response (Allen et al., 1995b; Shield, 2003).

The level of voluntary muscle activation (expressed as a percentage) was calculated using the following equation:

\[
\left\{ \frac{1 - \text{Superimposed response}}{\text{Control response}} \right\} \times 100 \\
\]

(Allen et al., 1995b)
The highest percentage across the three trials was recorded as the peak level of voluntary muscle activation whilst the average of the highest percentage in each trial was recorded as the mean level of voluntary muscle activation. To ensure consistency within and across the subjects, the acceptability of each trial was assessed immediately and the trial was repeated if any of the following characteristics were present:

- A plateau was less than 1.5 seconds in duration or no plateau was evident,
- The superimposed stimulus was administered over a rapidly increasing or declining force,
- Force levels continued to increase after the administering of a stimulus,
- The subjects did not perceive that they obtained a MVC.

(Shield, 2003)

3.3.4. VO₂ max Tests

An identical warm-up was completed prior to each test. The warm-up consisted of cycling at a workload of 100 watts and a cadence of 90 rpm for a period of five-minutes.

After completing the warm-up the subjects rested for three-minutes whilst sitting on the ergometer, over which time their pre-testing heart rate and expired respiratory gases were collected. The subjects then proceeded at a constant cadence of 90 rpm whilst the workload was increased by 25 watts each minute from an initial workload of 100 watts (Schell & Leelarthaepin, 1994). The test was terminated when the subjects could no longer maintain the designated workload or reached volitional exhaustion. The following criteria were used to assess if VO₂ max had been achieved:

- Oxygen uptake plateaued, that is when further increases in workload resulted in less than ±150 mL per minute increases in oxygen uptake.
- Respiratory exchange ratio was ≥ 1.15
- Blood lactate level exceeded 8 mmol/L
- Heart rate was equal to or in excess of the subject’s age predicted maximum
- Volitional exhaustion, that is a rating of perceived exertion ≥ 18

(Howley, Bassett, & Welch, 1995)
3.3.5. Discontinuous Incremental Cycling Efficiency Test

The control cycling efficiency (CE) tests and repeat tests were all completed on the same day of the week and at the same time of day across the study, to minimise the potential effects of biological rhythms (Reilly, Atkinson, & Waterhouse, 1997). The test-retest reliability of the newly developed discontinuous incremental cycling efficiency test was determined prior to the commencement of Study 1 (Appendix G). The results indicated high levels of test-retest reliability.

(i) Unloaded Cycling Test

For those tests that followed the MVC assessment, 10 minutes rest was allowed prior to commencing testing. Subjects completed an unloaded cycling test (absence of an external workload, i.e. power output = 0 watts) on a mechanically braked cycle ergometer prior to each cycling efficiency test (Nickleberry & Brooks, 1996; Sidossis, Horowitz, & Coyle, 1992). Before starting the test, the subjects sat on the cycle ergometer for a period of five minutes whilst pre-testing expired respiratory gases and heart rate were collected. After completing the rest period the subjects commenced pedalling with no load at a constant cadence of 90 rpm for a period of five-minutes. The data collected from the last three minutes of this period was used for the determination of the oxygen cost of unloaded cycling. The average minute unloaded cycling value was used for the calculation of corresponding level of energy expenditure (Hagberg, Mullin, Giese, & Spitznagel, 1981; Sidossis et al., 1992).

(ii) Loaded Cycling Test

Immediately following the unloaded cycling test the subjects completed a discontinuous incremental efficiency test consisting of three 10-minute bouts of cycling with two-minute rest intervals. The workloads corresponded to 20, 40 and 60% of VO\textsubscript{2} max, respectively. Steady state VO\textsubscript{2} and respiratory exchange ratio (RER) during the last five minutes of each stage, was used for the calculation of energy expenditure and efficiency.

3.4. Testing and Training Safety Precautions

- All subjects were required to warm-up prior to each strength testing and training session using both cycling exercise and a series of sub-maximal lifts.
• All strength testing and training sessions were supervised and spotters provided for each set, to ensure subject safety in view of the high-intensity of effort required.

• Mechanical stops were used to limit the ROM in all leg-press and bench-press exercises (both for training and testing) to safeguard against excessive ROM in the case of a failed attempt.

• The cycling ergometer in the hill cycling training sessions was secured to the treadmill guide rails via restraints, to prevent the cycle ergometer from moving whilst the subjects were riding the cycle ergometer (Figure 3.3).

• During each test session, the subject’s physical condition was constantly monitored to ensure safety.

### 3.5. Instrumentation and Materials

#### 3.5.1. Laboratory Facilities

All tests and training were carried out in the Exercise Physiology, Sports Conditioning and Rehabilitation Laboratories of the School of Exercise Science and Sport Management, Southern Cross University, Lismore. The Exercise Physiology Laboratory was accredited by the Australian Sports Commission, under the Laboratory Standards Assistance Scheme (LSAS), for oxygen consumption, blood analysis, ergometry and anthropometry (period 1998-2001).

All physiological testing was performed under controlled environmental conditions. The room temperature, barometric pressure and relative humidity for the respective experiments of Study 1 and Study 2 testing/training sessions are outlined in tables (Tables 3.1 and 3.2).
Table 3.1. Room temperature, barometric pressure and relative humidity of the VO₂ max test, control efficiency test (CE) and the efficiency tests post the strength training sessions in Study 1 (S = strength training, SE = strength endurance training, H = hill cycling training, SUL = upper and lower body strength training session) (Mean, brackets represent SD).

a) Experiment 1A

<table>
<thead>
<tr>
<th>Variable</th>
<th>VO₂ max</th>
<th>CE</th>
<th>S</th>
<th>SE</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature (°C)</td>
<td>22.7</td>
<td>22.9</td>
<td>23.1</td>
<td>22.9</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.6)</td>
<td>(0.5)</td>
<td>(0.6)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>Barometric Pressure (mmHg)</td>
<td>764</td>
<td>759</td>
<td>759</td>
<td>757</td>
<td>760</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(3)</td>
<td>(5)</td>
<td>(3)</td>
<td>(5)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>54.9</td>
<td>48.5</td>
<td>47.8</td>
<td>52.3</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>(7.5)</td>
<td>(7.4)</td>
<td>(5.8)</td>
<td>(11.3)</td>
<td>(8.9)</td>
</tr>
</tbody>
</table>

b) Experiment 1B

<table>
<thead>
<tr>
<th>Variable</th>
<th>VO₂ max</th>
<th>CE</th>
<th>S</th>
<th>SE</th>
<th>H</th>
<th>SUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature (°C)</td>
<td>22.7</td>
<td>23.0</td>
<td>23.2</td>
<td>23.1</td>
<td>22.9</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.7)</td>
<td>(0.5)</td>
<td>(0.7)</td>
<td>(0.4)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Barometric Pressure (mmHg)</td>
<td>765</td>
<td>758</td>
<td>759</td>
<td>757</td>
<td>760</td>
<td>758</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(3)</td>
<td>(5)</td>
<td>(3)</td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>56.6</td>
<td>47.8</td>
<td>48.4</td>
<td>51.2</td>
<td>48.2</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>(7.5)</td>
<td>(8.3)</td>
<td>(5.8)</td>
<td>(10.6)</td>
<td>(7.7)</td>
<td>(7.8)</td>
</tr>
</tbody>
</table>
Table 3.2. Room temperature, barometric pressure and relative humidity for the VO$_2$ max, control efficiency test (CE) and weights-cycle (WC) and cycle-weights (CW) endurance cycle (End.) and cycle efficiency tests (Eff.) in Study 2 (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>VO$_2$ max</th>
<th>CE</th>
<th>WC End.</th>
<th>WC Eff.</th>
<th>CW End.</th>
<th>CW Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature (°C)</td>
<td>23.1 (0.3)</td>
<td>23.3 (0.3)</td>
<td>23.2 (0.3)</td>
<td>23.6 (0.4)</td>
<td>22.8 (0.5)</td>
<td>23.2 (0.5)</td>
</tr>
<tr>
<td>Barometric Pressure (mmHg)</td>
<td>758 (4)</td>
<td>758 (5)</td>
<td>759 (4)</td>
<td>758 (5)</td>
<td>761 (5)</td>
<td>759 (5)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>40.6 (10.9)</td>
<td>46.0 (7.7)</td>
<td>52.2 (7.4)</td>
<td>49.0 (7.0)</td>
<td>50.0 (9.7)</td>
<td>43.2 (9.2)</td>
</tr>
</tbody>
</table>

3.5.2. Strength Training Equipment

The leg-press, bench-press and lat pull-down strength assessments and subsequent strength training sessions were completed on commercially available strength training machines (Figure 3.1) (Kolossal Fitness Systems, Sydney, Australia). The seat/bench, foot platform or bar height and safety stops were adjusted on all equipment to accommodate each subjects’ limb length.
3.5.3. Isometric Strength Testing Equipment

All MVC strength tests were carried out on a custom-built testing chair with a leg-extension lever arm set at an angle of 70° of knee flexion measured from full extension (Figure 3.2). The lever arm, with a moveable load cell attached to the distal end, was custom designed (Southern Cross University) and secured to the chair. A goniometer was used to set the knee angle to ensure a standardised position across the tests. The subjects were positioned in an upright-seated position (the same upright seat position with 90° of trunk flexion was used across all subjects). The subject’s right foot was secured to the lever arm via a velcro shin restraint attached to the load cell and was positioned just superior to the malleolus of the ankle. The subject was instructed to hold onto the bottom of chair during all trials for trunk stabilisation in addition to being secured into the chair by a five-point harness with straps over the shoulders, around the waist/hips and between the legs (Figure 3.2).
Maximal voluntary isometric contraction torque data was measured via a load cell at a sampling rate of 1000 Hz by a 12-bit analogue to digital converter connected to an Associative Measurement Laboratory (AMLAB) system (Associative Measurement Laboratories, Australia), which displayed and recorded trials in real-time. The system was calibrated prior to each test session by suspending a range of known weights from the load cell. All seat, load cell height, and computer settings were recorded for the isometric strength equipment to ensure standardized positioning across all MVC and muscle activation test sessions.

![Figure 3.2. Position for the MVC and muscle activation (twitch interpolation) tests.](image)

**3.5.4. Muscle Activation (Twitch Interpolation)**

The muscle activation tests were carried out whilst the subject was seated in the testing chair as described under “Isometric Strength Testing Equipment”, above (Figure 3.2). Two oval shaped (approximately 7.6cm x 12.7cm) self-adhering silver reusable surface electrodes (Uni-patch, Wabasha, USA) were used to administer the electrical stimuli provided via a constant current stimulator (Digitimer DS7AH, Hartfordshire, England). The electrodes were positioned transversely across the quadriceps femoris muscle at the following sites:
Cathode electrode - at the proximal, a third of the distance between the greater trochanter and femur lateral epicondyle,
Anode electrode - approximately 6 cm superior to the base of the patella.

(Shield, 2003)

The electrode sites were prepared by shaving the hair from the surface sites, cleansing the sites with an alcohol swab to remove surface oils then allowing the sites to dry before attaching the self-adhering electrodes.

The same AMLAB computer system, which displayed and recorded trials in real-time during the MVC trials, was used to record the muscle activation trials.

3.5.5. Cycle Ergometer

The VO$_2$ max and unloaded and loaded cycling efficiency tests were carried out on a mechanically braked cycle ergometer (Monark 868, Sweden), which was calibrated prior to each testing session by suspending known weights from the belt of the flywheel. During each test, cadence (rpm) and work output (watts) were monitored continuously using a custom-built electronic device and computer software system (Southern Cross University, Australia).

The endurance cycling training sessions in Study 2 were carried out on a Lode Excalibur Sport V2.0 cycle ergometer (Lode BV, Groningen, The Netherlands) in order that work output (watts) could be set to provide a constant workload independent of cadence.

For both ergometers, the seat height was adjusted for each subject's leg length, so as to allow each subject to have his preferred flexion of the knee joint when the pedals were in the bottom position. The handle bar height and position was also adjusted to accommodate each subject's trunk and arm length. The cycle ergometer set-up (seat and handle bar position) was recorded for each subject to ensure that a standardised position was maintained across each of the tests.

3.5.6. Treadmill

The hill cycling strength training sessions in Study 1 – Experiment 1A were carried out on a mechanically braked cycle ergometer, as described above, placed on a custom-built treadmill
(McKee Engineering, Lismore, Australia) raised to an incline of 10% (Figure 3.3). The workload was adjusted after the bike was placed on the treadmill incline so that the workload would not be increased by movement of the pendulum in raising the bike from the horizontal to incline positions. The incline was used to simulate a typical hill-climbing slope and provided similar biomechanical positioning to what the subjects would have experienced if they had been cycling on an actual hill outdoors (Swain & Wilcox, 1992).

![Figure 3.3](image.jpg)

**Figure 3.3.** Positioning of the cycle ergometer on the treadmill at a 10% incline for use during the hill cycle training sessions.

### 3.5.7. Respiratory Gas Analysis

Throughout the VO$_2$ max and unloaded and loaded cycling efficiency tests as well as the cycling endurance training sessions in Study 2, expired respiratory gases were collected at one-second intervals and averaged and reported at 15-second intervals via a custom-built computer software system (Southern Cross University, Australia). These gases were collected via an indirect calorimetry system consisting of;
- Hans Radolph 2700 two-way respiratory valve (Hans Rodalph Inc. U.S.A.),
- Morgan OA-ZR19 oxygen analyser (P.K. Morgan. U.K.),
- Ametek CD-3A carbon dioxide analyser (Ametek U.S.A) and

The gas analysis system was turned on at least one hour before commencing each test session to enable the analysers to stabilise. The gas analysis system was calibrated using three known alpha standard reference gases prior to each test. The measured parameters were:

- Temperature (°C),
- Barometric pressure (mmHg),
- Relative humidity (%),
- Fraction of oxygen in expired gas (FEO₂),
- Fraction of carbon dioxide in expired gas (FECO₂),
- Volume of air inhaled per minute (V₁) (L.min⁻¹) (ATPS), and
- Respiratory rate (br.min⁻¹).

The reported variables are:

- Volume of oxygen consumption per minute (VO₂) (mL.min⁻¹) (STPD),
- Volume of carbon dioxide production per minute (VCO₂) (mL.min⁻¹) (STPD),
- Volume of expired gas per minute (VE) (L.min⁻¹) (BTPS),
- Respiratory rate (RR) (br.min⁻¹) and
- Respiratory exchange ratio (RER).

3.5.8. Energy Expenditure and Efficiency Measures

The above reported variables, along with the thermal equivalent of oxygen for non-protein respiratory quotient (McArdle et al., 1996) were used to estimate energy expenditure for the respective periods in each of the cycling efficiency tests. The energy expenditure estimates were then substituted into the following equations in order to determine gross and net efficiency.

\[
\text{Gross Efficiency (GE)} = \frac{\text{Actual work completed}}{\text{Energy expended}}
\]
Net Efficiency (NE) = \frac{Actual \ work \ completed}{Energy \ expended – rest} \tag{Gaesser & Brooks, 1975}

Note: ‘rest’ refers to energy expended whilst sitting quietly on the cycle ergometer prior to exercise (Gaesser & Brooks, 1975; Kang et al., 1997).

3.5.9. Blood Specimen Collection

Two blood collection methods were used:

3.5.9.1. Finger-Prick (Study 1 and 2)

A capillary blood specimen was obtained via a small incision made on the skin from a Brand Safety Flow Lancet (Buton Dickinsin & Company, USA). Before the incision was made, the selected finger was cleansed with an alcoholic swab. The finger was then lanced and the first drop of blood wiped away using a tissue. A seventy-five microlitre capillary tube (heparinized) of blood was then collected and immediately transferred into 1.5 mL microfuge tubes of buffer (ratio 1:3) and frozen for future analysis of blood lactate concentration.

For those blood sampling times that corresponded with creatine kinase determination points, two additional 75 µL capillary tubes of capillary blood were collected. One end of the capillary tubes was sealed using clay (Cha Seal, Chase Instruments) before being centrifuged at 4000 rpm for five minutes at 4°C (Hettich Zentrifugen Universal 16R, Germany). The plasma was then extracted and placed into 1.5 mL microfuge tubes and frozen for future analysis.

3.5.9.2. Venipuncture (Study 2)

A 9 mL venous blood specimen was collected via a sterile plain vacuette tube (Greiner, Lubertechnik) by inserting a 21-gauge needle into the antecubital vein. Before inserting the needle, the site was cleansed with an alcoholic swab and a tourniquet applied above the puncture site. To assist with sampling, the subjects were asked to clench their fist during collection. On removal of the needle, the puncture site was covered with a sterile dressing and secured using tape. The sample was left to clot for 10 minutes before being centrifuged at
3200 rpm for 10 minutes (Phoenix orbital 300, Sydney, Australia). The serum was then aliquoted immediately to two microfuge tubes and stored at –80°C for future analysis of serum testosterone and cortisol concentration. All venous blood specimens were collected by a trained phlebotomist.

### 3.5.10. Blood Analysis

#### 3.5.10.1 Blood Lactate

The blood samples were left to return to room temperature before being analysed in duplicate using a YSI-1500 Sport L-lactate Analyser (Yellow Spring Instruments, U.S.A.) for determination of whole blood lactate (BL) concentrations. The analyser was calibrated prior to each session using four known concentration reference samples.

#### 3.5.10.2. Plasma Creatine Kinase

Aliquots of the plasma from the whole blood samples were left to return to room temperature before being analysed in duplicate by spectrophotometry using a Kodak Ektachem DTSC Module (Kodak Co, New York, U.S.A). Ten microlitres (10 μL) of plasma was deposited on a Kodak Ektachem DT CKMB slide (Johnson and Johnson Clinical Diagnostic Inc., New York, U.S.A) and inserted into the module for the determination of creatine kinase (CK). The module was calibrated prior to use using known references.

#### 3.5.10.3. Hormones

The Enzyme Linked Immunosobent Assay (ELISA) technique using a MRX Microplate Reader (Dynex Technologies, Germany) was used for the quantitative measurement of testosterone and cortisol concentrations in the serum samples. The individual procedure for assay of the respective hormones is outlined below.

(a) Testosterone Assay Procedure

The serum testosterone assay was completed in duplicate using a Fertigenix-Testo-Elisa kit (BIOSOURCE EUROPE S.A, Nivelles, Belgium). All reagents and samples were allowed to reach room temperature (~22°C) and mixed thoroughly by gentle inversion and vortex before use, respectively. Fifty microlitres (50 μL) of standards, controls and samples was pipetted to
the bottom of an anti-testosterone coated microtiter 96 well plate. Immediately following, 200 iL of testosterone-horseradish peroxidase (HRP) conjugate was added to all wells. The plate was then incubated for two hours at room temperature on a horizontal shaker at 600 rpm. Following incubation, the plate was washed by decanting the liquid from each well by inversion, before 0.4 mL of washing solution was pipetted into each well and again the contents of each well decanted. This washing procedure was repeated twice. Immediately following the final wash, 200 iL of revelation solution [tetramethylbenzydine (TMB) - Hydrogen Peroxide (H₂O₂)] was then added to each well and the plate incubated for a further 30 minutes at room temperature on a horizontal shaker set at 600 rpm. On cessation of shaking, 50 iL of stopping reagent (H₂SO₄ 1.8 N) was added to each well and the plate placed in the MRX Microplate Reader for reading at 450 nm. The amount of the substrate turnover was determined colorimetrically by measuring the absorbance, which is inversely proportional to the testosterone concentration. The mean of the duplicate samples was calculated, a standard curve plotted and the testosterone concentration of each sample determined by interpolation from the standard curve, using Revelation Software V.3.04 (Dynex Technologies, Germany). The intra-assay coefficients of variation were < 2%.

(b) Cortisol Assay Procedure

The serum cortisol assay was completed in duplicate using a Cortisol Elisa kit (BIOSOURCE EUROPE S.A, Nivelles, Belgium). All reagents and samples were allowed to reach room temperature (~22°C) and mixed thoroughly by gentle inversion and vortexing before use, respectively. Twenty micro litres (20 iL) of standards (0, 20, 50, 100, 200, 400 and 800 ng/mL) and samples were pipetted to the bottom of an anti-cortisol coated 96 well microplate. Immediately following, 200 iL of incubation solution (HRP-cortisol conjugate) was added to all wells. The plate was then incubated for 60 minutes at room temperature. Following incubation, the plate was washed by decanting the incubation solution from the wells by inversion, before 0.4 mL of washing solution was pipette into each well and again the contents of each well decanted. This washing procedure was repeated three times. After the last wash the inverted plate was placed on absorbent paper and gently tapped to remove any residual solution from the wells. Immediately following the final wash, 100 iL of chromogen/substrate mixture (TMB and H₂O₂) was pipette to each well and the plate incubated for a further 15 minutes at room temperature. The reaction was stopped by the addition of 100 iL of stopping reagent (0.5M H₂SO₄) into the wells. The plate was then
gently shaken before being read at 450 nm in the MRX Microplate Reader. The absorbance of the solution is inversely proportional to the cortisol concentration. The mean of the duplicate samples was calculated, a standard curve plotted and the cortisol concentration of each sample determined by interpolation from the standard curve, using Revelation Software V.3.04 (Dynex Technologies, Germany). The intra-assay coefficients of variation were < 5.3%.

3.5.11. Electrocardiograph

An electrocardiograph (ECG) unit (302 Rigel Cardiac Monitor, P.K. Morgan U.K.) was used to monitor heart rate (HR) and ECG continuously throughout the rest and exercise periods of the VO₂ and cycling efficiency tests as well as the cycling endurance training sessions in Study 2.

The electrodes were positioned in a modified Lead II configuration at the following sites:

Left Arm - 5 th intercostal space, mid-clavicular line on the left side;
Right Arm - slightly to the right side of the mid-clavicular line and slightly inferior to the clavicle on the right side;
Right Leg - mid-clavicular line just below the bottom rib on the right side.

(Southern Cross University, 2001)

The electrode sites were prepared by shaving the hair from the skin, abrading the skin to remove the outer epidermal layer, cleansing the site with an alcohol swab to remove surface oils then allowing the site to dry before attaching the self-adhesive electrodes (Red Dot, 3M Health Care, U.S.A.).

When the subject was seated on the cycle ergometer, the ECG leads were attached and secured in place using microporise tape (3M Health Care, U.S.A.). The subject’s heart rate was recorded at 15-second intervals throughout the tests by custom-built computer software (Southern Cross University, Australia).

3.5.12. Body Temperature Monitoring

A portable FirstTemp Thermometer (Model 2000A, Intelligent Medical Systems Inc, U.S.A) was used to measure tympanic temperature (TT). Disposable FirstTemp Genius probe covers
(Sherwood Medical Co., U.S.A) were used for each measurement. To limit the effect of clothing on body temperature regulation, subjects were required to cycle without a shirt and in short leg cycling pants only.

3.5.13. Rating of Perceived Exertion

The Borg rating of perceived exertion (RPE) 6-20 scale (Borg & Noble, 1974) was used to ascertain the subject’s perception of effort during the VO$_2$ max and cycling efficiency tests. The subject’s RPE score was recorded in the last 15 seconds of each minute during the VO$_2$ max tests and at the end of each stage during the cycling efficiency tests.

3.5.14. Height and Gross-Body-Mass

Subject’s GBM was measured prior to commencing the familiarisation session and VO$_2$ max tests. The pre-familiarisation session values were used to determine the warm-up and starting loads for the strength assessments, whilst the pre-VO$_2$ max test values were used to determine each subject’s relative VO$_2$ max. The mean height and mass outlined in the Subject section for each study reflects the values recorded prior to the commencement of the VO$_2$ max test.

Mass was measured on a calibrated electronic Mettler ID2 Multirange scale (August Sauter GmgH, West Germany), with the subject wearing only training shorts and recorded to the nearest 0.01 kg. Body height was measured using a wall-mounted stadiometer (Somatometre “Inter 16”) with the subject in bare feet and recorded to the nearest 0.01 m.

STUDY 3

3.6. Subject Preparation

All subjects were screened for health status and risk factors using a Pre-Participation Health Status Assessment Questionnaire (Appendix A) prior to participation. Each subject was provided with a detailed description of the purpose of the tests, the testing procedures and the risks involved with the study (Appendix D), and required to sign an informed consent form prior to participation. The experimental procedure was approved by the University of Copenhagen Ethics Committee.
Prior to each trial day, all subjects were required to:

1. Avoid participating in any exercise within 36 hours of the trial day.
2. Refrain from consuming any caffeine, supplements or drugs during the time of the dietary intake monitoring (refer below).
3. Refrain from consuming any alcohol within 24 hours of the trial day.

(Mahler et al., 1995)

To limit the possible impact of prior bouts of weight training undertaken by the subjects on the test results, the subjects were asked to refrain from participating in any training within 48 hours of each trial day.

In addition, because of the sensitive nature of some of the test variables to food and fluid intake, each subject was required to maintain a dietary intake diary from the day prior to the first control day, starting with the evening meal, until after the final blood sample on the day following the control day. To standardise the subjects’ dietary intake, the subjects were provided with a list of the types of foods from which they could prepare their meal, as well as the time allocated to the meal (19:00 hours). By standardising the time period for the evening meal and instructing the subjects to refrain from consuming any food or drink other than water after this time, a 12-hour fast was imposed prior to the morning blood sample on the control day. The evening meal, prepared the night prior to their first control day, was replicated for the evening meal of the first control day. Further, it was required that the subjects replicate this dietary intake for each subsequent period on the remaining control and sequence days.

3.6.1. Familiarisation Session

A familiarisation session was completed in the week prior to commencing testing and was completed using the same procedure as outlined in Study 1. Further, as was the case with Study 1 and 2, because of the subjects’ familiarity with strength training exercise, the familiarisation session was also used as the test time to determine each subjects’ leg-press 1 RM and leg-curl and extension 6 RM. These strength assessments were performed after 15 minutes rest following the completion of the cycle ergometer familiarisation trials.
3.7. Protocols

3.7.1. Muscular Strength Assessment (1 RM and 6 RM)

The leg-press 1 RM and leg-curl and -extension 6 RM muscular strength tests were used to evaluate each subject’s maximal level of strength in a single dynamic movement, in order to determine the loads (percentage of 1 RM or 6 RM) to be used in the W sessions. A 6 RM protocol was used for the leg-curl and -extension exercises instead of a 1 RM protocol, to help reduce the stress on the muscular and joint complexes of the knee that would be encountered using a 1 RM from a fully extended or flexed knee position.

Prior to commencing the test, the subjects warmed up on a cycle ergometer for five minutes at 1 watt per kg of GBM and performed light stretches of the major leg muscle groups (quadriceps, hamstrings and calves). Each subject then completed the strength test session using the same protocol as outlined in Study 1 and 2. The same protocol as used for the leg-press exercise (i.e. standardised warm-up trials with descending repetitions with progressively heavier loads) was also used for the leg-curl and -extension exercises.

3.7.2. VO2 max Tests

The VO2 max test was used to set the workload for the endurance cycling training sessions to ensure a standardised relative workload for each subject. The same testing protocol as used in Study 1 and 2 was employed.

3.7.3. Dietary Intake Monitoring

Immediately following the resting blood specimen collection on the morning of the first control day, the subjects consumed a high carbohydrate (CHO) breakfast. Each subject was supplied with the same type of food but free to consume the quantity of each item, as they felt necessary. The type and volume of food was recorded in order that the exact same type and quantity of food could be replicated for the second meal of the day and the quantity of CHO, protein and fat determined. Breakfast was consumed 50 minutes prior to the first training session. The second meal (lunch) on the control day was consumed 50 minutes prior to the time corresponding to the second training session time period and was a replicate of the
breakfast meal, as mentioned above. The subjects then replicated this dietary intake for each subsequent period on the remaining control and sequence days.

Breakfast on the sequence days occurred immediately following the resting muscle biopsy. In addition, on the mornings following the sequence days, breakfast and lunch were also provided (breakfast and breakfast/lunch for the WC and CW sequences, respectively) and were a replicate of the meals served on the trial days. The reason why breakfast and lunch were served for the CW sequence is that, the muscle biopsy taken 24 hours post the W session placed it at 16:00 hours. Consequently, to limit the possible influence of a change in diet on the muscle specimens, the same meals as those consumed on the CW sequence day were used.

During the cycling endurance training session for both the control and sequence days, the subjects ingested 5 g/100 mL glucose solution at a rate of 600 mL.h\(^{-1}\) to help maintain hydration whilst also providing a source of energy intake, given the duration of the C session. Fluid intake was ingested in 200 mL servings every 20 minutes throughout the ride (total volume ingested 1600 mL). Following the C session the subjects were also provided with a snack consisting of two small bananas, which equated to approximately 37 g of CHO.

The percentage of CHO, protein and fat consumed for all meals during the trial days was calculated as approximately 85, 10 and 5% and equated to 11, 1.5 and 0.3 g.kg\(^{-1}\) of GBM, respectively.

3.8. Instrumentation and Materials

3.8.1. Laboratory Facilities

All physiological tests and study C sessions were carried out in the laboratories of the Copenhagen Muscle Research Centre, Copenhagen, Denmark. The environmental conditions for the study VO\(_2\) max tests and C training sessions are provided in Table 3.3. The W sessions were completed in the strength training room of the Institute of Exercise and Sport Sciences, University of Copenhagen, Denmark.
Table 3.3. Room temperature, barometric pressure and relative humidity for the respective VO₂ max and C sessions during the control and sequence days (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>VO₂ max</th>
<th>C</th>
<th>WC</th>
<th>CW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature (°C)</td>
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<td>23.0</td>
<td>25.3</td>
<td>25.0</td>
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<td></td>
<td>(2.0)</td>
<td>(1.9)</td>
<td>(3.0)</td>
<td>(2.6)</td>
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<td>Barometric Pressure (mmHg)</td>
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<td>760</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(7)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>46.9</td>
<td>43.2</td>
<td>44.6</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>(12.7)</td>
<td>(6.9)</td>
<td>(8.2)</td>
<td>(6.1)</td>
</tr>
</tbody>
</table>

3.8.2. Strength Training Equipment

The leg-press, -curl and -extension strength assessments and subsequent W sessions were completed on commercially available strength training machines (Nordic Gym, Bollnas, Sweden) (Figure 3.4). The seat/bench, foot placements, lever length and safety stops were adjusted on all equipment to accommodate each subject’s limb length and recorded to standardise across all training sessions.
Figure 3.4. Strength training equipment used in the strength assessment and W sessions, (a) leg-press; (b) leg-curl and (c) leg-extension.
3.8.3. Cycle Ergometer

The VO₂ max test and C sessions were carried out on a Monark Ergomedic 839E (Monark Exercise AB, Vansbro, Sweden), which was calibrated prior to each testing session. During each test and training session, work output (measured in watts) was set to provide a constant workload independent of cadence. The seat height was adjusted for each subject's leg-length, so as to allow each subject to have their preferred flexion of the knee joint when the pedals were in the bottom position. The handle bar height and position was also adjusted to accommodate each subject's trunk and arm length. The cycle ergometer set-up was recorded for each subject to ensure that a standardised position was maintained across each of the tests.

3.8.4. Respiratory Gas Analysis

Throughout the VO₂ max and study C sessions, expired respiratory gases were collected and analysed continuously via a Med Graphics CPX/D Exercise System with Med Graphics CPX Series 2 computer software (Medical Graphics Corporation, St Paul, U.S.A.). These gases were collected via an indirect calorimetry system consisting of;

- Hans Radolph 2700 series two-way NRBV respiratory valve (Hans Rodalp Inc., Kansas City, U.S.A.),
- Clinical Pulmonary Function Spirometry System CPF-S

The gas analysis system was turned on at least one-hour before commencing each test/training session to enable the analysers to stabilise. The gas analysis system was calibrated using two known standard reference gases prior to each test. The measured parameters and reported variables are the same as those for Study 1 and 2.

3.8.5. Energy Expenditure and Efficiency Measures

The same methods were used to estimate energy expenditure and gross and net efficiency for the respective periods of the C sessions, as described for Study 1 and 2.
3.8.6. Blood Specimen Collection and Analysis

3.8.6.1. Blood Lactate

One-millilitre blood specimens were obtained using a 1 mL QS50 pre-heparinized syringe (Radiometer Medical A/S, Copenhagen, Denmark) from a three-way BD connector attached to a 20-gauge indwelling cannula (Becton Dickinson, Helsingborg, Sweden) inserted in the antecubital vein. Before collection, 1 mL of blood was withdrawn using a plain 5 mL syringe (Becton Dickinson SA, Madrid, Spain) and discarded. The blood was discarded because the cannula was flushed after every specimen was collected and at 20-minute intervals throughout the control and sequence days using 1 mL of heparin treated saline solution (1 µL heparin per 1 mL saline, Sygehus Apotekerne i Danmark, Copenhagen) to prevent the cannula from clotting. Immediately following collection, 125 µL of the specimen was injected into an EML™ 105 Electrolyte Metabolite Laboratory Analyser (Radiometer Danmark A/S, Denmark), for analysis of whole BL concentration. The analyser was calibrated prior to each specimen collection time point using known reference samples.

3.8.6.2. Hormones

Five millilitre venous blood specimens were obtained at each time point using a 10 mL plain syringe (ONCE, Codan Medical Aps, Denmark) from a three-way BD connector attached to a 20-gauge indwelling cannula (Becton Dickinson, Sweden) inserted in the antecubital vein. Before collection, 1 mL of blood was withdrawn using a plain 5 mL syringe (Becton Dickinson SA, Spain) and discarded, as outlined in the blood lactate collection method above (Note: for the those time points when blood lactate and hormone specimens coincided, the blood lactate specimen was collected first followed immediately by the hormone specimen). Immediately following collection, the whole blood specimen was transferred to a 15 mL plain glass tube treated with ethylenediaminetetraacetic acid (EDTA, 10 µL per 1 mL whole blood). The tube was then gently inverted to mix the contents before being centrifuged at 4000 rpm for five minutes at 4°C (Eppendorf refrigerated centrifuge 5810R, Germany). Following this, the plasma was pipetted to 3.6 mL Nunc CryoTube Vials (Nagle Nunc International, Denmark) and stored at −20°C for future analysis of plasma testosterone and cortisol concentration levels.
After the last control and sequence day specimen was collected the cannula was removed. Consequently, the following morning’s venous blood specimen was collected using a venipuncture technique, as used in Study 2. The site was cleansed with an alcoholic swab and a tourniquet applied above the puncture site before the venous blood was drawn into two sterile 3 mL plain vacuette tubes (BD Vacutainer, Becton Dickinson, U.K). The contents of the vacuette tubes were transferred to plain glass tubes treated with EDTA, and the procedure completed as described above. To assist with sampling, the subjects were asked to clench their fist during collection.

3.8.6.3. Hormone Assay Procedure

The radioimmunoassay (RIA) technique using a Wallac 1470 Wizard automatic gamma counter (Wallac Denmark AS, Denmark) was used for the quantitative measurement of testosterone and cortisol concentrations in the plasma specimens. The individual procedure for assay of the respective hormones is outlined below.

(a) Cortisol

The plasma cortisol samples were assayed in duplicate using a DSL-2100 ACTIVE™ Cortisol Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories Inc., U.S.A). All reagents and samples were allowed to reach room temperature (approximately 22-25°C) and mixed thoroughly by gentle inversion and vortex before use, respectively. Plain uncoated plastic tubes were used for Total Count tubes whilst Anti-Cortisol-Coated Tubes were used for standards, controls and samples. Twenty-five micro litres (25 iL) of standards, controls and samples were transferred to the bottom of the respective tubes. Immediately following, 500 iL of the Cortisol [I-125] Reagent was added to all the tubes, the tubes capped and the samples shaken gentle by hand in the racks to ensure the reagent and standards, controls and samples were mixed. All tubes including standards and controls were then incubated in a warm bath (Jouan, Type J18 Bain Universal, France) at 37 ± 2°C for 45 minutes. On removal from the bath, all the tubes excluding the Total Count Tubes were decanted by simultaneous inversion. The tube racks were sharply struck before being placed on absorbent paper for two minutes to facilitate complete drainage. The tubes were then re-capped and placed in the automatic gamma counter for one minute for counting. The mean of the duplicate samples
was calculated, a standard curve plotted and the cortisol concentration of each sample determined by interpolation from the standard curve.

(b) Testosterone

The plasma testosterone samples were assayed in duplicate using a DRG Testosterone (ct) I-1
25 Radioimmunoassay Kit (DRG Diagnostics, Germany). Before starting the assay procedure, the lyophilised standards were reconstituted by adding 1.0 mL of distilled water to the 0 mg/mL standard and 0.5 mL to the remaining standards. All reagents and samples were allowed to reach room temperature (approximately 22-25°C) and mixed thoroughly by gentle inversion and vortex before use, respectively. Plain uncoated plastic tubes were used for Total Count tubes whilst Anti-Testosterone-Coated Tubes were used for standards, controls and samples. After 25 iL of standards, controls and samples were pipetted to the bottom of the respective tubes, 1.0 mL of Testosterone$^{125}$I Reagent was added to all the tubes, the tubes capped and the samples vortexed briefly to ensure the reagent and standards, controls and samples were mixed. All tubes including standards and controls were then incubated in a warm bath (Jouan, Type J18 Bain Universal, France) at 37°C for 120 minutes. On removal from the bath, all the tubes excluding the Total Count Tubes were decanted by simultaneous inversion. The tube racks were sharply struck before being placed on absorbent paper for two minutes to facilitate complete drainage. The tubes were then re-capped and placed in an automatic gamma counter for one-minute for counting. The mean of the duplicate samples was calculated, a standard curve plotted and the testosterone concentration of each sample determined by interpolation from the standard curve.

3.8.7. Muscle Biopsies

3.8.7.1. Sampling Technique

Whilst the subjects were lying supine, muscle specimens were taken using the percutaneous needle biopsy technique with suction, obtained from the middle portion of the vastus lateralis muscle (Pilegaard et al., 2000). The same procedure was used for all specimens. The site was cleansed using a guaze swab and antiseptic (Klorhexidinsprit Farvet 0.5%, HIS Apoteket, Denmark) before 20-30 mL of local anaesthetic (Lidokain-Andrenalin SAD, Sygehus Apotekerne i Danmark, Copenhagen) was injected into the tissue, fascia and muscle around
the site. Using a sterile surgical blade, a small incision of approximately 10 mm was made longitudinally along the muscle through the skin and fascia. The biopsy needle, with a 75 mm length plastic connection tubing (Clinco, Hersfeld, Denmark) inserted into the hollow of the biopsy needle plunger with a 60 mL syringe (ONCE, Codan Medical Aps, Denmark) attached, was then inserted into the muscle through the fascia to a depth of approximately 20 mm. The plunger of the biopsy needle was then withdrawn at which time the syringe plunger was also withdrawn, providing suction of the muscle tissue into the biopsy needle aperture. The biopsy plunger was then used to cut the specimen, before the needle was removed. The specimen, approximately 100 mg, was then immediately placed on sterile gauze swabs briefly to absorb any excess blood before being placed in liquid nitrogen (N₂). The specimens were then transferred to pre-cooled 1.8 mL Nunc Cryotube Vials (Nagle Nunc International, Denmark) and stored at −80°C for future dissection and analysis of muscle glycogen content and gene expression levels.

Following each biopsy, the wound was closed using sterile dressing strips (3M Health Care, USA) and covered with a wound dressing to allow the subjects to exercise. Even though multiple specimens were required over the course of each sequence day, a new incision was made in the vastus lateralis muscle for each specimen. Specimens were taken from both the right and left legs and where possible pre- and post-specimens for a given training session were taken from the same leg. The sampling sites were positioned approximately 15 mm apart longitudinally and laterally.

**3.8.7.2. Muscle Specimen Dissection**

From the muscle biopsy specimens, two pieces were cut, one of 5-10 mg and the other 25-30 mg for the determination of muscle glycogen content and muscle gene expression levels, respectively. The muscle glycogen specimens were placed in small custom made plastic aerated freeze dry containers (University of Copenhagen, Denmark) ready for freeze drying whilst the gene expression specimens were placed in 1.8 mL Nunc Cryotube Vials (Nagle Nunc International, Denmark) ready for RNA isolation. All dissections were carried out in a Cryostat chamber (Caravel Model 327-427, Anders Brøndum AS, Denmark) and the specimens weighed using an electronic Metler UMT2 scale (Melter Toledo S/A, Denmark). All specimens were kept on dry ice during transportation and whilst awaiting cutting. The specimens were then stored at −80°C until analysed.
3.8.8. Muscle Glycogen Content

On removal from storage, the specimens were freeze-dried in a custom-built freeze drier (University of Copenhagen, Denmark) for a period of 48 hours. Following this, the specimens were removed from the freezer and attached to the drier to return to room temperature conditions overnight. The freeze-dried jars were then sealed before removal from the machine and transferred to a controlled environmental room (temperature 19.9°C and 24% humidity) for dissection. Before dissection, a specimen was removed from the freeze dry jar, weighed using an electronic scale (Model UMT2, Melter Toledo S/A, Denmark) and left on the scale until the weight stabilized. Each specimen’s weight was then recorded before being placed under a microscope (Model SZ40, Olympus, Japan) where all blood, debris and non-muscle tissue were removed. The specimens were then re-weighed and their weight recorded before being placed in 4.5 mL Nunc vials (Nagle Nunc International, Denmark). The specimens were then hydrolysed by adding 1 mL of hydrochloric acid (HCl, Bie-Berntsen AS, Denmark) to all vials and the specimens boiled for two hours. The specimens were then refrigerated at 4°C before the glycogen content was determined by a standard enzymatic (hexokinase) UV technique (ABX Diagnostics-Parc Euromedicine, France) using a Cobas Fara II detection system (F.Hoffmann-La Roche, Switzerland) within 24 hours.

3.8.9. RNA Isolation Technique

The procedure for the isolation of total RNA was a modified guanidinium thiocyanate (GT)-phenol-chloroform extraction method adapted from Pilegaard et al (2000). The procedure for all genes investigated was identical, as outlined below.

3.8.9.1. Guanidinium Thiocyanate Solution Preparation

Before the isolation procedure was performed, the GT solution was prepared. A hundred millilitres (100 mL) of GT solution was made by adding 47.26 g of guanidine thiocyanate to 50 mL of diethyl pyrocarbonate (DEPC) treated water (H₂O) whilst stirring on low heat. Once completely dissolved, 20 mM of sodium acetate (NaOAc, in DEPC H₂O) and 0.5 g N-lauryl-sarcosine was then added and the total volume brought to 90 mL using DEPC H₂O. The solution was then brought a pH of 5.0 with 1M NaOH before adjusting the total volume to 100 mL using DEPC H₂O. The solution was then filter-sterilised using a disposable nylon
filtration unit (Nalge Nunc International, U.S.A.) and transferred to DEPC-treated brown bottle before being autoclaved.

3.8.9.2. Isolation Procedure

Each frozen muscle biopsy specimen of approximately 25-30 mg was added to 14 mL polypropylene tubes (Becton Dickinson, U.S.A), pre-cooled (-80°C) in liquid nitrogen (N₂). Two millilitres of GT was added to each tube individually and homogenised using a Polytron PT 2100 (Kinematica AG, Luzern) for 20 seconds [Note: After homogenising, all tubes/samples were kept on ice (-20°C) whilst awaiting processing]. Following homogenisation, total RNA was extracted by the addition of 200 ìL of 2M NaOAc (pH 4.0 in DEPC H₂O), 2 mL of DEPC H₂O saturated phenol and 500 ìL of chloroform isoamyl-OH (49:1). Tubes were capped and shaken vigorously for 15 seconds. The samples were then placed on ice (-20°C) for 15 minutes before being centrifuged at 12,000 g for 20 minutes at 4°C (Eppendorf refrigerated centrifuge 5810R, Germany). This separated the sample into an aqueous and organic layer. Two millilitres of the aqueous layer was transferred by pipette to 5 mL polypropylene tubes (Nalge Nunc International, U.S.A.), to which 5 ìL of yeast tRNA was added to facilitate localization of the RNA pellet before 2 mL of cold isopropanol was added to precipitate the total RNA from the aqueous phrase. The samples were then vortexed, placed at -20°C for 15 minutes before centrifugation at 12,000 g for 10 minutes at 4°C to pellet the RNA. The supernatant was then poured off and the tube placed upside down to drain. Five hundred micro litres (500 ìL) of GT solution was then added to the tubes before being left on ice for 10 minutes. The solution was then transferred to sterile, RNase free 1.5 mL microfuge tubes, (Eppendorf, Germany) to which 500 ìL of ice-cold isopropanol was added before being vortexed and placed at -20°C for 15 minutes. On removal, the samples were centrifuged at 12,000 g for 10 minutes at 4°C to pellet the RNA. The supernatant was then poured off and 1 mL of cold 75% ethonal (EtOH) in DEPC H₂O added before being vortexed gently to rinse the pellet. The sample was then centrifuged at 12,000 g for 5 minutes at 4°C before the supernatant was poured off, the pellet vacuum dried and resuspended in 2 ìL of DEPC H₂O containing 0.1 mM EDTA, per mg of muscle. The pellet was then left to dissolve overnight at 4°C.
3.8.9.3. Reverse Transcription Reaction

First strand cDNA synthesis was performed on RNA samples using the Superscript II RNase H system (GIBCO-BRL, Invitrogen Life Technologies, U.S.A) (Hildebrandt & Neufer, 2000). After the RNA pellets were left to dissolve overnight at 4°C, the samples were removed and 11 µL of each sample was transferred to 0.5 mL microfuge tubes (Eppendorf, Germany). One microlitre (1 µL) of Oligo dT was added, vortexed gently before being placed at 70°C for 10 minutes (Stuart Scientific block heater, U.K.). The samples were then chilled on ice (-20°C) for 5 minutes before a cocktail of 4 µL of first strand buffer, 2 µL of DTT and 1 µL of dNTP was added to each sample and placed at 42°C for two minutes (Techne Dri-block DB-2D, U.K). One micro litre (1µL) of Superscript was then added, vortexed gently followed by incubation at 42°C for 50 minutes. The samples were then placed at 70°C for 15 minutes to heat inactivate the reaction before being placed at -20°C whilst awaiting analysis. The total volume of the cDNA sample was 20 µL.

3.8.9.4. RNA Analysis

Each of the cDNA samples was thawed and diluted using 150 µL of filter-sterilised H2O to give a total volume of 170 µL. From this 5.5 µL of each cDNA sample was mixed with a cocktail consisting of a Taqman Universal PCR Master Mix (Applied Biosystems, U.S.A.), forward and reverse primers, a probe and H2O to give a total volume of 55 µL. The Taqman Master Mix consisted of AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs, magnesium chloride, primers and probes and Taqman PCR Buffer (Note: the volume of Master Mix per sample was held constant across all genes however, the volume of probe, forward and reverse primers and H2O varied according to the optimisation previously carried out in the lab on the respective genes). Each sample was then vortexed briefly before 15 µL was pipetted to the bottom of 3 wells in an ABI Prism 96-well optical reaction plate (Applied Biosystems, U.S.A.). All 12 samples for each subject, plus a “No Template Control” and “Control” sample, were included on the same plate. Two genes were run on each plate at a time. Transparent micro-amp optical caps (Applied Biosystems, U.S.A.) were used to seal the wells before the plate was placed in a real-time Polymerase Chain Reaction (PCR) machine, ABI PRISM 7700 Sequence Detection System (Applied Biosystems, U.S.A.), to quantify the expression level of each gene in the samples.
A total of 17 genes was analysed, 9 metabolic and 6 muscle growth, grouped loosely on the basis of their overall function (Table 3.4). The remaining two genes were endogenous controls or “housekeeping genes” as they are often referred. These genes were analysed in order to provide an active reference because it is not possible to accurately quantify the amount of RNA in each sample following the RT process and as such different quantities of cDNA may be present in each sample. However, by using an endogenous control and quantifying the amount of this gene present in each sample, it is possible to normalise quantify (i.e. relative quantitation) the amount of a selected gene in a sample. The two common endogenous genes used by researchers for the quantitation of gene expression are β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Both β-actin and GAPDH were analysed but only GAPDH was used in this study because it was more stable during exercise of the type and intensity performed in this study compared to β-actin.

The relative quantitation method used to determine the quantity of RNA in each sample, was by an arithmetic formula where the amount of RNA for the selected gene, normalised to the endogenous reference and relative to a calibrator, was given by:

\[ 2^{-\Delta\Delta C_{T}} \]

Where:

\( \Delta C_{T} \) refers to the difference between the selected genes cycle and the endogenous reference’s cycle (in this case GAPDH), at the given threshold (T).

\( \Delta\Delta C_{T} \) refers to the difference between the \( \Delta C_{T} \) value and the \( \Delta C_{T} \) value of the calibrator. The calibrator refers to the sample used for comparative purposes and in this study referred to the pre-training sample taken at 07:00 hours at the beginning of each sequence day. Consequently, the quantity of RNA in each sample was expressed as an \( n \)-fold difference relative to the pre-training sample, which was set to 1 (Applied Biosystems, 1997).
Table 3.4. List of genes analysed, grouped according to their overall function.

<table>
<thead>
<tr>
<th>Function</th>
<th>Gene</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic</td>
<td>Pyruvate dehydrogenase kinase 4</td>
<td>PDK4</td>
</tr>
<tr>
<td></td>
<td>Proliferator-activated receptor gamma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>coactivator-1</td>
<td>PGC1</td>
</tr>
<tr>
<td></td>
<td>Lipoprotein lipase</td>
<td>LPL</td>
</tr>
<tr>
<td></td>
<td>Carnitine palmitoyltransferase</td>
<td>CPT1</td>
</tr>
<tr>
<td></td>
<td>Hexokinase II</td>
<td>HKII</td>
</tr>
<tr>
<td></td>
<td>Uncoupling protein 3</td>
<td>UCP3</td>
</tr>
<tr>
<td></td>
<td>Glycogen synthase</td>
<td>GYS</td>
</tr>
<tr>
<td></td>
<td>Lactate dehydrogenase-M</td>
<td>LDH-M</td>
</tr>
<tr>
<td></td>
<td>Lactate dehydrogenase-H</td>
<td>LDH-H</td>
</tr>
<tr>
<td>Muscle Growth</td>
<td>Insulin-like growth factor 1 &amp; 2</td>
<td>IGF1 &amp; 2</td>
</tr>
<tr>
<td>Sarcomeric proteins</td>
<td>Myosin heavy chain I</td>
<td>MHCI</td>
</tr>
<tr>
<td></td>
<td>Myosin heavy chain IIa</td>
<td>MHCIIa</td>
</tr>
<tr>
<td></td>
<td>Myosin heavy chain IIx</td>
<td>MHCIIx</td>
</tr>
<tr>
<td>Myogenic regulatory factors</td>
<td>MyoD</td>
<td>MyoD</td>
</tr>
<tr>
<td></td>
<td>Myogenin</td>
<td>Myogenin</td>
</tr>
</tbody>
</table>

3.8.10. Electrocardiograph and Heart Rate Monitor

An ECG unit (Diascope 1, S & W Medico, Albertslund, Denmark) was used to monitor heart rate and ECG continuously throughout the rest and exercise periods of the VO₂ max tests and C sessions. The sites were prepared and the electrodes positioned in a modified Lead II configuration, as described in Study 1.

The subject’s heart rate data was recorded at five-second intervals throughout the test and exercise sessions by a Polar Vantage NV (Polar Electro Danmark APS, Denmark).

3.8.11. Height and Gross-Body-Mass

Each subject’s height and GBM was measured as described for Study 1 and 2.
3.9. Test-Retest Reliability

The degree of test-retest reliability of any variable measured or protocol is dependent upon the two main sources of variation – biological variation and technical error. To limit the possible influences of biological variation and technical error across all three studies, due to the multiple days of testing:

- All tests were undertaken in controlled environmental conditions, as previously outlined in the Instrumentation and Materials section (Laboratory Facilities).
- All equipment was calibrated prior to each testing session using known standard references.
- The same ergometer, testing equipment and laboratory analysis equipment were used across all tests.
- There was a minimum of three days between the familiarisation session, VO₂ max test and the first trial day and a minimum of seven days between each of the trial days, in order to eliminate any possible carry over effects from one test to another.
- Further to the above point, all subjects were tested on the same day of the week and at the same time of day for the respective trial days in Study 1, 2 and 3, one week apart (two weeks for the sequence days in Study 3), over the test period in order to limit the influence of varying test times (eg. morning to afternoon) or the influence of different meal patterns.
- Each subject was motivated to give 100% effort at each test/training session via verbal encouragement.
- The subjects were allowed to train in between the weekly testing sessions however, they were asked not to alter their weekly training regimes (i.e. frequency, intensity or duration) throughout the period of their participation in the study.
CHAPTER 4

Study 1

The acute effects of strength training on the recovery of muscle force generating capacity and cycling efficiency post-training

4.1. Introduction

Investigations into the development of strength have identified that the intensity and duration of training are key factors in determining the acute physiological responses (Atha, 1981; Feigenbaum & Pollock, 1997; McDonagh & Davies, 1984). Prior investigations have also highlighted that the mode of exercise and the type of muscle contraction (i.e. isometric, concentric or eccentric) affect the level of fatigue experienced and rate of recovery following exercise (Byrnes, Clarkson, White et al., 1985; Fridén, 1984; Kroon & Naeije, 1991; McCully & Faulkner, 1985; Newham, 1988; Sargeant & Dolan, 1987; Stauber, 1989). Whilst the acute effects of prior endurance training on muscle force-generating capacity have been investigated (Bentley et al., 1998; Sherman et al., 1984), it remains unknown as to the acute effect of varying intensities, duration and modes of strength training on subsequent endurance performance.

The overall aim of Study 1 was to examine the acute residual physiological effects of strength training of different intensities (high and low), durations (60 and 30 minutes) and modes (weight lifting and hill cycling) on subsequent muscle force generating capacity and cycling efficiency three hours post-training and blood variables three and 24 hours post-training. Specifically,

- do high-intensity strength training sessions have greater residual physiological effects than low-intensity strength training sessions when equated for work volume?
• do longer duration high-intensity strength training sessions have greater residual physiological effects than lesser duration high-intensity strength training sessions?

• do traditional strength training sessions incorporating both eccentric and concentric contractions have a greater physiological effect than hill cycling strength training, which incorporates concentric contractions only?

4.2. Methods

4.2.1. Study Design

Study 1 consisted of two experiments:

4.2.1.1. Experiment 1A

Following a familiarisation session, as described in the General Methodology and Materials Chapter (Section 3.2.2), each subject participated in five experimental sessions over a five-week period. The first two sessions were used as control sessions (VO₂ max test and a discontinuous incremental cycling efficiency test), followed by a session of lower body strength (S), a session of lower body strength endurance (SE) and a session of hill cycling (H) training.

On each training day the subjects attended the laboratory to provide a blood specimen, before undertaking an isometric strength/muscle activation test 10 minutes prior to carrying out a bout of strength training. Three hours after the completion of the strength training the subjects again completed an isometric strength/muscle activation test followed 10 minutes later by the same discontinuous incremental cycling efficiency test as used for the control day. Twenty-four hours after the pre-strength training blood collection time point, the subjects returned to the laboratory to provide another blood specimen (Figure 4.1).

The control day was a duplicate of the training days (Figure 4.1) except that no actual training sessions or isometric strength/muscle activation tests were completed. The control efficiency (CE) test was performed at the same time of the day as the training day tests.
The subjects were randomly allocated to one of the three testing orders, with three subjects completed in each order.

Order 1. S SE H
Order 2. SE H S
Order 3. H S SE

There was a minimum of three days between the familiarisation session, VO$_2$ max test and CE test and seven days between the CE test and each of the strength training sessions, in order to eliminate any possible carry-over effects from one test to another.

4.2.1.2. Experiment 1B

Seven of the subjects from Experiment 1A went on to complete a fourth training protocol consisting of both upper and lower body strength training (SUL), the week following the completion of Experiment 1A. The fourth training protocol was included to examine the impact of a longer duration (60 minutes) high-intensity training protocol on the same physiological variables as those of the three shorter duration (~30 minutes) strength training protocols. The same experimental design was used as described above for Experiment 1A (Figure 4.1).
Training and Muscle Activation Schedule

Cycling Efficiency Test Control Day

Experiment 1A and 1B Training Days

Blood Sampling Schedule

Figure 4.1. Study Design – Experiment 1A and 1B: An example of the control and training days’ time lines. The dark shaded areas refer to the strength training sessions (S, SE, H or SUL), respectively whilst the light shaded area refers to the cycling efficiency test. The large and small arrows reflect the muscle activation and blood sampling time points, respectively. The final small arrow for the “blood sampling schedule” refers to the 24 hours after the pre-strength training sample.
4.2.2. Subjects

4.2.2.1. Experiment 1A

A total of nine (9) male athletes who were actively participating in cycling and strength training on a regular basis, volunteered for the study. Their mean (±SD) age, height, body mass and VO₂ max (absolute and relative) are outlined in Table 4.1.

Table 4.1. Descriptive characteristics of the nine subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>(±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.77</td>
<td>0.06</td>
</tr>
<tr>
<td>Body Mass (kilograms)</td>
<td>80.28</td>
<td>12.03</td>
</tr>
<tr>
<td>VO₂ max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (L.min⁻¹)</td>
<td>4.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Relative (mL.kg⁻¹.min⁻¹)</td>
<td>50.98</td>
<td>7.67</td>
</tr>
</tbody>
</table>

The subjects were selected according to the following criteria.

- Aged between 18 and 40
- Currently cycling a minimum of 2-3 times per week with an approximate weekly duration total of 2-3 hours
- A minimum of 12 months cycling and weight training experience
- Currently performing lower and upper body resistance exercises a minimum of two times per week
- No contraindications to the testing procedure

4.2.2.2. Experiment 1B

The mean (±SD) age, height, body mass and VO₂ max (absolute and relative) of the seven (7) subjects who went on from Experiment 1A and completed the fourth training protocol are outlined in Table 4.2.
Table 4.2. Descriptive characteristics of the seven subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>(±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.79</td>
<td>0.04</td>
</tr>
<tr>
<td>Body Mass (kilograms)</td>
<td>84.43</td>
<td>10.11</td>
</tr>
<tr>
<td>VO\textsubscript{2} max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (L.min(^{-1}))</td>
<td>4.20</td>
<td>0.40</td>
</tr>
<tr>
<td>Relative (mL.kg(^{-1}).min(^{-1}))</td>
<td>49.96</td>
<td>7.94</td>
</tr>
</tbody>
</table>

4.2.3. Strength Training Protocols

After completing the isometric strength/muscle activation test on each of the training days in Experiment 1A and 1B, the subjects rested for 10 minutes before completing one of the following protocols.

**Strength (S) Training Session**

**Warm-up:** Five minutes of stationary cycling at a relative workload corresponding to 1 watt per kg of GBM followed by two sub-maximal trials at 30 and 60% of 1 RM with descending repetitions of ten and five.

**Training Sets:** Leg-press 6 x 6 RM

The 6 RM was calculated as 85% of each subject’s 1 RM (Feigenbaum & Pollock, 1997; Hoeger et al., 1990)

**Rest Intervals:** One-minute between warm-up trials and three minutes between training sets

**Duration:** ~30 minutes
Strength Endurance (SE) Training Session

Warm-up: Five minutes of stationary cycling at a relative workload corresponding to 1 watt per kg of GBM followed by two sub-maximal trials at 25% of 1 RM for 12 repetitions.

Training Sets: Leg-press 6 x 20 repetitions
The load for the 20 repetitions was equated to the amount of work completed by each subject during their S training sets. This corresponded with a load equal to 25% of each subject’s 1 RM.

Rest Intervals: One-minute between warm-up trials and three minutes between training sets

Duration: ~30 minutes

Hill Cycling (H) Training Session

Warm-up: Five minutes of stationary cycling at a relative workload corresponding to 1 watt per kg of GBM followed by two 20-second sub-maximal trials at twice the work completed for the S and SE training warm-up sets.

Hill Grade: 10% incline provided by a treadmill (Figure 3.3) in order to simulate a typical hill climb used by cyclists for strength training (Swain & Wilcox, 1992)

Cadence: 50 rpm

Hill Repetitions: Six repetitions at a workload corresponding to twice the work completed by each subject during their S and SE training sets (note: twice the work of the S and SE training sets was used for this protocol in preference to reducing the time limit to ten
seconds for each rep or increasing the workload to unrealistic levels)

**Time per Rep:** 20 seconds with five seconds lead-in time

**Rest Intervals:** One-minute between warm-up trials and three minutes between training sets

**Duration:** ~30 minutes

**Upper and Lower Body Strength (SUL) Training Session**

**Warm-up:** Five minutes of stationary cycling at a relative workload corresponding to 1 watt per kg of GBM followed by two sub-maximal trials at 30 and 60% of leg and bench press 1 RM and lat pull-down 6 RM for ten- and five-repetitions, respectively.

**Training Sets:**
- Leg-press 6 x 6 RM (same load as per S training session)
- Bench-press 4 x 6 RM
- Lat pull-down 4 x 6 RM

The 6 RM loads were calculated as 85% of each subject’s bench- and leg-press 1 RM (Feigenbaum & Pollock, 1997; Hoeger et al., 1990) and 100% of each subject’s lat pull-down 6 RM.

**Rest Intervals:** One-minute between warm-up trials and three minutes between training sets

**Duration:** ~60 minutes

**4.2.4. Sampling Protocols and Measurements**

The specimens and variables collected or measured during the control day and training days for both Experiment 1A and 1B, respectively are outlined below.
4.2.4.1. Maximal Voluntary Isometric Contraction Assessment

In order to establish if there was a difference between the strength training sessions of different intensities, durations and modes, with respect to post-strength training MVC force (i.e. differences in muscle force-generating capacity), each subject performed three single leg-extension trials pre and three hours post-training, as described in the General Methodology and Materials Chapter (Section 3.3.2). Maximal voluntary contractions were used, as they have been established as an acceptable and reliable form of strength evaluation (Bemben, Massey, Boileau, & Misner, 1992; Hortobágyi & Lambert, 1992).

4.2.4.2. Muscle Activation (twitch interpolation)

During the MVC session each subject was also assessed for voluntary and involuntary muscle activation using a twitch interpolation technique, a technique widely accepted in the assessment of muscle activation (Allen et al., 1995b; Behm et al., 1996; Belanger & McComas, 1981; Gandevia, 2001). This technique involved applying increasing levels of electric shocks to the quadriceps femoris muscle and observing whether force levels increased, as described in the General Methodology and Materials Chapter (Section 3.3.3). By applying an electrical stimulus during a voluntary contraction and immediately after when the muscle was relaxed, the level of muscle activation was determined. This method was used for the determination of whether a peripheral or central fatigue component contributed to the isometric strength results in the three hours post-strength training (Allen et al., 1995b; Gandevia, 2001; Shield, 2003).

4.2.4.3. Blood Specimen Collection

Capillary blood specimens were collected from the fingertip at the following time periods:

- pre and immediately (< 30 seconds) after the VO2 max tests,
- pre and immediately (< 30 seconds) after each strength training session,
- pre, during (at the end of each stage) and immediately (< 30 seconds) after each cycling efficiency test, and
- 24 hours after the pre-strength training session specimen,
for the determination of resting, exercising (cycle efficiency test) and post-exercise

- blood lactate concentration, and
- plasma CK activity. (*

(* = Not applicable to VO₂ max tests)

Blood lactate concentration was assessed, as changes in lactate concentration following a bout of exercise have been shown to adversely influence exercise performance performed after an initial bout of exercise (Brooks et al., 2000). Creatine kinase was assessed because it is found almost exclusively in muscle tissue and is considered a marker of the extent of muscle damage following a training session (Ebbeling & Clarkson, 1989; Sayers, Clarkson, & Lee, 2000). All blood specimens were collected using the finger-prick technique.

4.2.4.4. Discontinuous Incremental Cycling Efficiency Test

The purpose of the cycling efficiency test was to provide a repeatable standardised test to examine the effects of the strength training protocols on physiological responses to exercise. Throughout the stages of the cycling efficiency tests (i.e. rest, unloaded, 20, 40 and 60% of VO₂ max workloads) a number of variables (including blood specimens as outlined above) were collected or measured (respiratory gases, heart rate, ECG, tympanic temperature and RPE). The specimens and variables were collected and analysed as described in the General Methodology and Materials Chapter (Section 3.5). The workloads for the cycling efficiency test were determined from the VO₂ max test completed in the week prior to the cycling efficiency test control day. The same variables were measured during the VO₂ max test as for the cycling efficiency tests except for tympanic temperature.

4.2.4.5. Respiratory Gases

Even though expired respiratory gases were collected continuously during the rest, unloaded and loaded stages of the cycling efficiency test, only the last five minutes of each stage were used for reporting purposes. Apart from reporting the respiratory variables, oxygen uptake, ventilation and respiratory rate, the respiratory gases were also used to estimate energy expenditure for the respective sampling periods from which gross and net efficiency were
calculated for the three loaded workload stages, as described in the General Methodology and Materials Chapter (Section 3.5.8).

4.2.4.6. Heart Rate

Heart rate and ECG were monitored continuously throughout the cycling efficiency test and reported for the same time periods as outlined for the respiratory variables above.

4.2.4.7. Body Temperature Monitoring

Tympanic temperature was measured in the last 30 seconds of each stage of the efficiency test.

4.2.4.8. Gross-Body-Mass

The subject’s GBM was measured prior to and immediately after each cycling efficiency test to determine GBM loss during the cycling efficiency tests.

4.2.4.9. Rating of Perceived Exertion

The subject’s RPE score was recorded in the last 15 seconds of each stage during the unloaded and loaded stages of the cycling efficiency test.

4.2.5. Statistical Analysis

Due to the similarity of the study design for Experiment 1A and 1B, the statistical procedures outlined below apply to both experiments unless otherwise indicated. Descriptive statistics (mean and SD) were calculated for all measured variables.

4.2.5.1. Comparison of Strength Training Variables

A three-factor repeated measures ANOVA was completed with two-within subject factors, training protocol (S, SE and H) and time (pre- and post-training) and one-between subject factor, test order. This analysis was completed for the dependent variables BL concentration and CK. The same analysis was completed for Experiment 1B except that the within subject factor, training protocol also included the SUL training protocol.
4.2.5.2. Comparison of the Maximal Voluntary Contraction and Muscle Activation Responses Pre and Post the Strength Training Protocols

The same analysis as that outlined above for the Comparison of the Strength Training Variable was used. This analysis was completed for the dependent variables peak and mean torque and muscle activation.

4.2.5.3. Comparison of the Efficiency Test Variables Post the Strength Training Protocols

In light of the number of subjects in Experiment 1A (nine) yet large number of protocols (four) and periods for the efficiency test (five: rest, unloaded, 20, 40 and 60%) the analysis was too large for analysis using a single ANOVA. Therefore, two three-factor repeated measures ANOVAs were completed with two-within subject factors, protocol (S, SE, H and CE) and period and one-between subject factor, test order. The within-subject factor period was split into two: (one) rest and unloaded cycling periods and (two) 20, 40 and 60% of VO\(_2\) max workload periods. Hence the first ANOVA included period one and the second ANOVA, period two. This analysis was completed for the dependent variables HR, RR, \(\bar{V_e}\), VO\(_2\), TT and BL concentration. A single three-factor (protocol by period by test order) repeated measures ANOVA was completed for the variables GE, NE, GBM and RPE where there were a reduced number of periods.

The same split analysis was completed for the efficiency test variables in Experiment 1B except that the within subject factor, protocol also included the SUL training protocol.

The Pearson’s Product Moment Correlation Coefficient was used to measure the degree of association between GE and NE and VO\(_2\) max at each workload period during the efficiency tests in Experiment 1B.

In the event of a violation of the sphericity assumption for any of the dependent variables, the Greenhouse-Geisser correction was used. For all analyses that indicated a significant effect, Bonferroni adjusted multiple pair-wise comparisons were completed to locate the source of the difference. An alpha level of .05 was used to indicate a level of significant difference for
all ANOVA analysis and a level of significant correlation for correlation analysis. All statistical analysis was completed using the Statistical Package for Social Sciences (SPSS version 11.0 for windows, SPSS Inc.).

4.3. Results – Experiment 1A

4.3.1. Effect of Test Order

The between-subject factor (test order) was included in all variable statistical analysis undertaken, however no effect of test order was found for any of the strength training, MVC, muscle activation or efficiency test variables measured (p>.05). To avoid unnecessary repetition of this finding within each variable result, a table of the statistical results are provided in Appendix F.

4.3.2. Comparison of the Strength Training Variables

4.3.2.1. Blood Lactate Concentration

A significant main effect of both training protocol (p<.001) and time (p=.002) was found. Post-hoc analysis indicated a significant difference between the post-training BL concentrations for the S, SE and H protocols (p=.002), with the S (4.85 ± 1.79 mmol.L⁻¹) protocol recording a significantly higher post-training BL concentration than the SE (p=.010, 2.41 ± 1.02 mmol.L⁻¹) and H (p=.001, 2.72 ± 1.48 mmol.L⁻¹) protocols (Figure 4.2, top). There was no significant difference between pre-training BL concentrations for any of the training protocols (p>.05). Significant changes in BL concentration were found from pre- to post-training for the S (p<.001), SE (p=.002) and H (p=.008) protocols. A significant training protocol by time interaction (p=.005) was also found indicating that the influence of the training protocol on the BL response depended on the time of sampling.

4.3.2.2. Creatine Kinase Activity

The S protocol recorded a higher post-training CK value (293.5 ± 147.8 U.L⁻¹) than the SE (197.0 ± 66.9 U.L⁻¹) and H (203.3 ± 111.7 U.L⁻¹) protocols (Figure 4.2, bottom), but the difference was not significant (p=.194). There was also no main effect of time found (p=.111) even though the S protocol showed a notable CK response compared to the SE and H protocols from pre- to post-training. A significant (p=.003) interaction between training
protocol and time was found indicating that the CK response was influenced by the type of training protocol but depended on the time of sampling.

![Blood Lactate and CK graphs](image)

**Figure 4.2.** Pre- and post-training BL concentration (top) and CK activity (bottom, n = 7) for the S, SE and H protocols (Mean, error bar represents SD).

* : Significantly different to pre (p<.05),
# : Significantly different to SE and H (p<.05)
4.3.3. Comparison of Maximal Voluntary Contraction and Muscle Activation Responses Pre and Post the Strength Training Protocols

4.3.3.1. Maximal Voluntary Contraction Peak and Mean Torque

Even though a significant main effect for training protocol (p=.026) was found, post-hoc analysis did not identify any significant difference between the training protocols for pre- or post-peak torque (p>.05). There was however, a significant main effect for time found (p<.001). Post-hoc analysis revealed that the S (p=.037) and H (p=.027) protocols recorded significantly greater reductions in peak torque (5.08% and 4.01%, respectively) when the respective reductions are expressed as a percentage of the pre-values, compared to the SE protocol (Table 4.3), which recorded a non-significant reduction of 3.50% (p=.202).

No significant main effect for training protocol was found for mean torque however, it did approach significance (p=.074). A significant main effect for time was found across the three training protocols (p<.001). Post-hoc analysis revealed that the S protocol recorded a significantly greater reduction in mean torque (6.15%, p=.010), when the respective reductions are expressed as a percentage of the pre-values, compared to the SE (2.39%) and H (4.22%) protocols (Table 4.3), which recorded non-significant reductions (p>.05). No interaction of training protocol by time was found for either peak (p=.931) or mean torque (p=.448).
Table 4.3. Pre- and post-training MVC peak and mean torque for the S, SE and H protocols (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Training Protocol</th>
<th>Peak Torque (Nm)</th>
<th>Mean Torque (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Strength</td>
<td>174.21</td>
<td>165.37*</td>
</tr>
<tr>
<td></td>
<td>(26.27)</td>
<td>(22.50)</td>
</tr>
<tr>
<td>Strength Endurance</td>
<td>182.75</td>
<td>176.35</td>
</tr>
<tr>
<td></td>
<td>(21.50)</td>
<td>(29.16)</td>
</tr>
<tr>
<td>Hill Cycling</td>
<td>179.53</td>
<td>172.33*</td>
</tr>
<tr>
<td></td>
<td>(25.05)</td>
<td>(21.05)</td>
</tr>
</tbody>
</table>

*: Significantly different to pre (p<.05)

4.3.3.2. Muscle Activation Peak and Mean Response

Comparison of the peak and mean pre- and post-training muscle activation (MA) responses for each protocol (Table 4.4), revealed no significant difference between the S, SE and H protocols (p=.582 and p=.286, respectively). Compared to pre-MA responses, there was no significant difference in peak (p=.436) or mean (p=.459) post-training responses for the three training protocols. No interaction between training protocol and time was found for either peak (p=.248) or mean MA (p=.371).
Table 4.4. Pre- and post-training peak and mean muscle activation responses for the S, SE and H training protocols (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Training Protocol</th>
<th>Peak Response (%)</th>
<th>Mean Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Strength</td>
<td>89.28 (6.14)</td>
<td>89.59 (5.97)</td>
</tr>
<tr>
<td>Strength Endurance</td>
<td>90.23 (5.39)</td>
<td>89.38 (4.63)</td>
</tr>
<tr>
<td>Hill Cycling</td>
<td>92.47 (3.63)</td>
<td>89.16 (7.02)</td>
</tr>
</tbody>
</table>

4.3.4. Comparison of the Efficiency Test Variables Post the Strength Training Protocols

Whilst a significant main effect of protocol was found for heart rate (HR) for the workload period comparison (p=.002) but not the rest or unloaded cycling periods (p=.104), no significant difference was found for respiratory rate (RR, p=.150 and .159), ventilation (VE, p=.193 and .420), oxygen uptake (VO₂, p=.405 and .507), BL concentration (p=.407 and .800), tympanic temperature (TT, p=.879 and .791), gross efficiency (GE, p=.781), net efficiency (NE, p=.227), GBM (p=.224) or RPE (p=.077), respectively. Even though the no main effect of protocol was found for the majority of the efficiency test variables, the S protocol consistently showed greater changes than the other two training protocols compared to the CE test, as outlined below.

Heart Rate - Post-hoc analysis revealed that the S protocol HR response was significantly (p<.05) greater than the SE and H protocols at the 20, 40 and 60% of VO₂ max workloads (Figure 4.3). There was no significant difference between the S and CE protocols even though the average increase in HR over the duration of the efficiency test for the S protocol above that of the CE was 4%. There was no significant difference between the SE and H protocols and the CE test (p>.05).
**Respiratory Responses** - the S protocol tended to have higher responses for all three variables (RR, $V_E$ and $VO_2$), particularly at the 40% and 60% of $VO_2$ max workloads than the SE and H protocols compared to the CE test. Minimal difference was found between the SE and CE tests for any of respiratory variables (Figure 4.4).

**Gross and Net Efficiency** – even though all protocols showed similar response patterns over the duration of the efficiency tests (Figure 4.5), the S protocol showed marginally lower GE and NE than the other two protocols as well as the CE test. GE and NE were nearly identical between the SE and CE tests whilst the H protocol showed an average increase in NE of 2% and similar GE as the CE test.

**Blood Lactate Concentration** - the S protocol showed an elevated BL concentration above that of the CE of 12% over the duration of the test, with the greatest difference occurring at both the 40% and 60% of $VO_2$ max workloads (~17%). Minimal difference was found between the CE and the SE (<1%) and H (<3%) protocols (Figure 4.3).

**Tympanic Temperature** - all protocols showed a similar response over the duration of the efficiency test, with no real change in TT from rest to 40% of $VO_2$ max workload, followed by an increase in TT from the 40 to 60% of $VO_2$ max workloads. The S protocol showed a slightly higher TT across the majority of the efficiency test periods than the other protocols and the CE test. There was however, some variability in the subjects’ responses (n = 8).

**Gross-Body-Mass** - a greater reduction in GBM occurred for the S protocol (0.38 ± 0.15 kg) than for the SE (0.28 ± 0.12 kg) and H (0.28 ± 0.12 kg) protocols, which showed similar reductions to that of the CE test (0.26 ± 0.13 kg) (Table 4.5).

**Rating of Perceived Exertion** - the majority of subjects indicated a higher RPE for the 40% and 60% of $VO_2$ max workloads for the efficiency test post the S protocol than for the SE, H or CE tests (Figure 4.6). Higher RPE were also found for the SE and H protocols compared to the CE test for the same workloads as found for the S protocol.
A significant main effect of time was found for all variables for the workload period comparisons (p<.05). However only HR, RR, V\textsubscript{E} and VO\textsubscript{2} showed a significant main effect of time for the rest and unloaded cycling period comparison (p<.001). A significant interaction of protocol by period was found for the variables GBM (p<.001) and RPE (p=.047), indicating that the influence of the type of protocol on the change in GBM and RPE was dependent upon the time of measurement. No interaction of protocol by period was found for the remaining test variables for either the rest and unloaded cycling or workload period comparisons (p>.05).
Figure 4.3. Heart rate response (top) and BL concentration (bottom, n = 7) for the rest and unloaded cycling periods and the 20, 40 and 60% of VO₂ max workload periods of the efficiency test for the CE, S, SE and H protocols (Mean, error bars represent SD).

* : S protocol significant greater than SE and H protocols (p<.05)
# : Significantly greater than the previous period (p<.05)
† : Significantly greater than 40% of VO₂ max workload period (p<.05).
Figure 4.4. Respiratory rate (top), $V_E$ (middle) and $VO_2$ (bottom) during the rest and unloaded cycling periods and the 20, 40 and 60% of $VO_2$ max workload periods of the efficiency test for the CE, S, SE and H protocols (Mean, error bars represent SD).

* : Significantly greater than the previous period (p<.05)
**Figure 4.5.** Gross (top) and net efficiency (bottom) during the 20, 40 and 60% of VO₂ max workload periods of the efficiency test for the CE, S, SE and H protocols (Mean, error bars represent SD).

* : Significantly greater than 20% of VO₂ max workload (p<.05)
# : Significantly greater than 40% of VO₂ max workload (p<.05).
Table 4.5. Gross body mass pre and post the cycling efficiency test for the CE, S, SE and H protocols (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th></th>
<th>CE</th>
<th></th>
<th>S</th>
<th></th>
<th>SE</th>
<th></th>
<th>H</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>GBM (kg)</td>
<td>79.98</td>
<td>79.72*</td>
<td>80.49</td>
<td>80.11*</td>
<td>80.21</td>
<td>79.93*</td>
<td>80.61</td>
<td>80.32*</td>
</tr>
</tbody>
</table>

*: Significantly different to pre (p<.05)

Figure 4.6. Rating of perceived exertion during the unloaded cycling period and the 20, 40 and 60% of VO\textsubscript{2} max workload periods of the efficiency test for the CE, S, SE and H protocols (Mean, error bars represent SD).

*: Significantly greater than 20% of VO\textsubscript{2} max workload (p<.05)
#: Significantly greater than 40% of VO\textsubscript{2} max workload (p<.05).
4.4. Results - Experiment 1B

4.4.1. Effect of Test Order

Seven subjects that completed Experiment 1A went onto complete Experiment 1B (the inclusion of the SUL training protocol), the week following the completion of the test orders in Experiment 1A, therefore it was deemed unnecessary to include the between-subjects factor test order within the statistical analysis, given that no effect of test order was found for Experiment 1A. Consequently, all statistical analysis was carried out excluding the between-subjects factor test order.

4.4.2. Comparison of the Strength Training Variables

4.4.2.1. Blood Lactate Concentration

The SUL protocol (7.29 ± 1.17 mmol.L⁻¹) recorded a significantly higher post-training BL concentration than the S (p=.019, 5.01 ± 1.92 mmol.L⁻¹), SE (p=.009, 3.00 ± 1.01 mmol.L⁻¹) and H (p=.002, 3.32 ± 1.75 mmol.L⁻¹) protocols (Figure 4.7, top). Further, the S protocol showed a significantly higher post-training BL concentration than the H protocol (p=.029). No significant difference was found between the SE and H protocols. All training protocols showed a significant (p<.05) increase in BL concentration from pre- to post-training. Whilst there was no significant difference between pre-training BL concentrations for any of the training protocols, there was a significant interaction of protocol by time (p<.001) indicating that the influence of the type of training on the lactate response was dependent upon the time of sampling. The results are based on five subjects.

4.4.2.2. Creatine Kinase

No significant difference between the four training protocols (p=.790) was found, even though the S and SUL protocols recorded higher post-training CK values than the SE and H protocols (Figure 4.7, bottom). There was however, a significant main effect of time (p=.021). Post-hoc analysis indicated that compared to pre CK levels, there was a significant increase in post-training levels in respect to the S (p=.002) and SUL (p=.035) protocols but not for the SE (p=.275) or H (p=.415) protocols. There was also a significant interaction of protocol by time (p=.019) indicating that the influence of the type of training on the CK response was dependent upon the time of sampling.
Figure 4.7. Pre- and post-training BL concentration (top, n = 5) and CK activity (bottom) for the S, SE, H and SUL training protocols (Mean, error bar represents SD).

* : Significantly different to pre (p<.05)
# : Significantly greater than S, SE, and H post (p<.05)
† : Significantly greater than H post (p<.05)
4.4.3. Comparison of Maximal Voluntary Contraction and Muscle Activation Responses Pre and Post the Strength Training Protocols

4.4.3.1. Maximal Voluntary Contraction Peak and Mean Torque

Even though a significant main effect of training protocol (p=.037) was found for peak torque, post-hoc analysis did not identify any significant (p>.05) differences between the four training protocols (Table 4.6). However, there was a significant main effect of time (p=.002), with the SUL (p=.019, 5.73%) and H (p=.020, 5.02%) protocols recording significantly greater reductions in peak torque, when the respective reductions are expressed as a percentage of the pre-values, compared to the S (p=.117, 4.06%) and SE (p=.398, 2.85%) protocols.

No significant main effect of training protocol (p=.115) was found for mean torque, but like peak torque, there was a significant main effect of time (p=.003). Post-hoc analysis revealed that the S (p=.047, 5.07%), H (p=.040, 6.05%) and SUL (p=.038, 5.40%) protocols all recorded significant changes in mean torque from pre- to post-training, when the respective reductions are expressed as a percentage of the pre-values, compared to the SE (p=.593, 1.98%) protocol. No interaction of training protocol by time was found for either peak (p=.842) or mean torque (p=.360).

4.4.3.2. Muscle Activation Peak and Mean Response

No significant main effect of training protocol (p=.490 and p=.179) or time (p=.420 and p=.337) was found for peak or mean MA, respectively (Table 4.7). In addition, no interaction of training protocol by time (p=.360 and p=.529) was found for either peak or mean MA responses, respectively. Even though statistical analysis did not indicate a significant difference between training protocols for mean MA results, it is worth noting that there was variability between subjects for the training protocols. For example, one subject recorded a 6.04% increase in MA for the SE training protocol and a 2.98% reduction for the SUL training protocol. In contrast, another subject recorded a 5.82% and 10.62% reduction in MA for the SE and SUL training protocols, respectively.
Table 4.6. Pre- and post-training MVC peak and mean torque (top) and peak and mean MA responses (bottom) for the S, SE, H and SUL training protocols (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Training Protocol</th>
<th>Peak Torque (Nm)</th>
<th>Mean Torque (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Strength</strong></td>
<td>177.85</td>
<td>170.63</td>
</tr>
<tr>
<td></td>
<td>(19.76)</td>
<td>(16.34)</td>
</tr>
<tr>
<td><strong>Strength Endurance</strong></td>
<td>187.70</td>
<td>182.36</td>
</tr>
<tr>
<td></td>
<td>(15.24)</td>
<td>(24.30)</td>
</tr>
<tr>
<td><strong>Hill Cycling</strong></td>
<td>185.25</td>
<td>175.96*</td>
</tr>
<tr>
<td></td>
<td>(17.30)</td>
<td>(11.17)</td>
</tr>
<tr>
<td><strong>SUL</strong></td>
<td>187.76</td>
<td>177.00*</td>
</tr>
<tr>
<td></td>
<td>(15.38)</td>
<td>(15.47)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Training Protocol</th>
<th>Peak Response (%)</th>
<th>Mean Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Strength</strong></td>
<td>88.91</td>
<td>88.86</td>
</tr>
<tr>
<td></td>
<td>(7.03)</td>
<td>(6.48)</td>
</tr>
<tr>
<td><strong>Strength Endurance</strong></td>
<td>88.83</td>
<td>88.94</td>
</tr>
<tr>
<td></td>
<td>(5.18)</td>
<td>(4.55)</td>
</tr>
<tr>
<td><strong>Hill Cycling</strong></td>
<td>92.13</td>
<td>88.13</td>
</tr>
<tr>
<td></td>
<td>(3.94)</td>
<td>(7.54)</td>
</tr>
<tr>
<td><strong>SUL</strong></td>
<td>88.78</td>
<td>86.75</td>
</tr>
<tr>
<td></td>
<td>(5.94)</td>
<td>(6.67)</td>
</tr>
</tbody>
</table>

*: Significantly different to pre (p<.05)
4.4.4. Comparison of the Efficiency Test Variables Post the Strength Training Protocols

A significant main effect of protocol was found for HR (p=.003) and VO$_2$ (p=.039) for the workload period comparison and HR for the rest and unloaded cycling period comparison (p=.050), whilst VO$_2$ approached significance (p=.065). The variable RR also approached significance for the rest and unloaded cycling period (p=.081) and workload period comparisons (p=.095) along with RPE (p=.091). No significant difference was found between the protocols for VE (p=.285 and .228), TT (p=.993 and .247), GE (p=.429), NE (p=.515) or GBM (p=.673) for the rest and unloaded cycling or workload period comparisons, respectively. Statistical analysis was not completed for BL due to technical difficulties with one or more of the sample time points for three of the subjects.

A significant main effect of time was found for all variables for the rest or unloaded cycling and workload period comparisons (p<.05) except RR, which showed no significant change across the three-workload periods (p=.095). A significant interaction of protocol by period was found for GBM (p<.002) and TT but only for the workload period comparison (p=.011), indicating that the influence of the type of protocol on the change in GBM and TT was dependent upon the time of measurement. No interaction of protocol by period was found for the remaining test variables for either the rest and unloaded cycling or workload period comparisons (p>.05).

Even though no main effect of protocol was found for the majority of the efficiency test variables, the SUL protocol consistently showed greater changes than the other training protocols compared to the CE test.

**Heart Rate** - Post-hoc analysis showed that the HR response for the S protocol was significantly (p<.05) greater than the SE protocol at the 20, 40 and 60% workloads (Figure 4.8). Even though post-hoc analysis did not indicate a significant difference between the remaining protocols, the average increase in HR over the duration of the efficiency tests for the S and SUL protocols, above that of the CE test, was 4.2% and 5.7%, respectively. In contrast, the SE and H training protocols resulted in a reduction of 2.9% and 0.5% compared to the CE test, respectively.
**Respiratory responses** – post-hoc analysis revealed a significant difference between the SUL and SE protocols at the 60% of VO₂ max workload period for VO₂ (p=.041). Whilst no significant difference was found between the SUL protocol and the CE test, the average increase in RR, Vₑ and VO₂ above that of the CE test, over the duration of the efficiency tests was 7.5, 5.8 and 6.3%, respectively compared to the SE and H protocols which showed similar responses to that of the CE test for all of the respiratory variables (Figure 4.9). The respiratory responses for the S protocol above that of the CE test were less than that of the SUL protocol showing an average increase of ~3% over the duration of the efficiency tests.

**Gross and Net Efficiency** – all protocols showed similar response patterns over the duration of the efficiency tests (Figure 4.10) however, the SUL protocol showed lower GE and NE than the other protocols including the CE test. The H protocol showed an average increase in NE of ~3% and similar GE as the CE test, whilst the SE and CE tests showed similar values for GE and NE.

Even though there was no significant difference between protocols for GE or NE, there was a notable difference between subjects across the protocols. For example: one subject recorded 13.01 and 11.29% GE for the CE and SUL protocols at the 20% of VO₂ max workload, increasing to 20.33 and 20.18% at the 60% of VO₂ max workload, respectively. In contrast, another subject recorded 8.28 and 7.92% GE for the CE and SUL protocols at the 20% of VO₂ max workload, increasing to 18.70 and 16.04% at the 60% of VO₂ max workload, respectively. Because of the differences between the subjects, a Pearson’s Product Moment correlation analysis was performed between the GE and NE results and VO₂ max for each workload across the protocols. A significant (p<.05) correlation was found between the majority of the efficiency test 20 and 40% of VO₂ max workloads and all the protocols for GE (average correlation r=.91 and r=.89) and NE (average correlation r=.92 and r=.73), respectively. The relationship weakened at the 60% of VO₂ max workload for both GE (average correlation r=.63) and NE (average correlation r=.49) but was still strong for the S and SUL protocols for GE and NE (r=.77-.85).

**Blood Lactate Concentration** - whilst the overall pattern of change over the duration of the efficiency test was very similar for the four training protocols (Figure 4.8), the S and SUL protocols resulted in the greatest increase in BL concentration above that of the CE with 23.4 and 22.8%, respectively. The most notable differences occurred at the 20 to 60% of VO₂ max
workloads. In contrast, the SE and H protocols had notably less impact compared to the CE protocol with 2.6 and 15.4% average increase, respectively.

**Tympamic Temperature** - the SUL protocol resulted in a higher TT being recorded across each of the efficiency test periods compared to the other four protocols, with the greatest difference occurring for the workload periods. The SUL (p=.028) and S (p=.032) protocols were the only protocols to show a significant increase in tympanic temperature from the 20 to 60% workload periods. Similar responses were found for the remaining protocols across the majority of the efficiency test periods.

**Gross-Body-Mass** - a greater reduction in GBM occurred for the SUL (0.52 ± 0.19 kg) and S protocols (0.41 ± 0.16 kg) than for the SE (0.30 ± 0.13 kg) and H (0.31 ± 0.13 kg) protocols, which showed similar reductions to that of the CE test (0.28 ± 0.14 kg) (Table 4.7).

**Rating of Perceived Exertion** - the majority of subjects indicated a higher RPE for the 40% and 60% of VO₂ max workloads for the efficiency test post the SUL and S protocols than for the SE, H or CE tests. Higher RPE were also found for the SE and H protocols compared to the CE test for the same workloads as found for the SUL and S protocols.
**Figure 4.8.** Heart rate (top) and BL concentration (bottom, \( n = 4 \)) for the rest and unloaded cycling periods and the 20, 40 and 60\% of VO\(_2\) max workload periods of the efficiency test for the CE, S, SE, H and SUL protocols (Mean, error bars represent SD).

* : S protocol significantly greater than SE protocol (\( p<.05 \))

# : Significantly greater than previous period (\( p<.05 \))
Figure 4.9. Respiratory rate, $V_E$ and $VO_2$ for the rest and unloaded cycling periods and the 20, 40 and 60% of $VO_2$ max workload periods of the efficiency test for the CE, S, SE, H and SUL protocols (Mean, error bars represent SD).

* : Significantly different to previous period (p<.05)
# : SUL protocol significantly greater than SE protocol (p<.05).
Figure 4.10. Gross and net efficiency during the 20, 40 and 60% of VO$_2$ max workload periods of the efficiency test for the CE, S, SE, H and SUL protocols (Mean, error bars represent SD).

* : Significantly greater than 20% workload (p<.05)
# : S, SE and H protocols, significantly greater than 40% workload (p<.05),
Table 4.7. Gross body mass pre and post the cycling efficiency tests for the CE, S, SE, H and SUL protocols (Mean, brackets represent SD)

<table>
<thead>
<tr>
<th></th>
<th>CE</th>
<th>S</th>
<th>SE</th>
<th>H</th>
<th>SUL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>GBM (kg)</td>
<td>83.93</td>
<td>83.65*</td>
<td>84.43</td>
<td>84.02*</td>
<td>84.19</td>
</tr>
<tr>
<td></td>
<td>(9.80)</td>
<td>(9.75)</td>
<td>(9.86)</td>
<td>(9.79)</td>
<td>(9.63)</td>
</tr>
</tbody>
</table>

*: Significantly different to pre (p<.05)

4.4.5. Summary of Results

4.4.5.1 Experiment 1A

In summary, a significantly higher post-training BL concentration was found for the S protocol compared to the SE and H protocols. Post-training CK levels also showed a similar trend. No significant difference was found between the three training protocols for peak or mean torque. However, a significant reduction in MVC mean torque was found three hours following the S protocol but not the SE or H protocols. A greater physiological cost of cycling was found during the efficiency test following the S protocol as shown by a significantly higher HR, higher BL concentrations, respiratory responses and TT, as well as greater reductions in GBM, GE and NE compared to the efficiency test following the SE and H protocols or CE test.

4.4.5.2 Experiment 1B

In summary, a significantly greater post-training BL concentration was found for the SUL protocol compared to the SE, H and S protocols. Further, the S protocol showed a significantly higher BL concentration than the H protocol. Whilst no significant difference was found between the four training protocols for CK levels, a significant increase in CK was found following the S and SUL protocols but not the SE and H protocols. No significant difference was found between the four training protocols for mean MVC torque or MA. However, the SUL, S and H protocols all recorded significant reductions in mean torque when the respective reductions are expressed as a percentage of the pre-values, compared to the SE
protocol. A similar result was also found for peak torque but only for the SUL and H protocols. The SUL protocol and to a lesser extent the S protocol showed a consistently higher physiological cost of cycling than the SE and H protocols compared to the CE with higher HR, BL concentrations, respiratory responses and TT, as well as greater reductions in GBM, GE and NE.
4.5. Discussion Overview

Due to the similarity in the design of the three studies for this thesis, with each study examining the effect of various training variables (intensity, duration, mode or sequence of training) on cycling efficiency and muscle recovery capacity in the hours post-training, a number of common factors were identified as contributing to the results. The discussion of these common factors has been combined in the General Discussion (Chapter 7) and as such will not be addressed during the discussion section of the individual studies. The common factors include the mechanisms contributing to,

- the reduction in force generating capacity and MA post the various strength training protocols in Study 1 and the two training sequences in Study 2; and

- the increased physiological cost and changes in GE and NE during the efficiency tests and C sessions of the training sequences in Study 1, 2 and 3, respectively.

The practical implications of the findings for the three studies will also be addressed following the Summary and Conclusion in Chapter 8.

4.6. Discussion

4.6.1. Introduction

The overall aim of Study 1 was to examine the acute residual physiological effects of strength training of different intensities, durations and modes of training, on muscle force generating capacity and cycling efficiency three hours post-training. The major findings of this study indicate that training variables, intensity, duration and mode of training are important factors in determining the acute residual physiological effects post-training. However, the variables differ in their effects between the recovery of force generating capacity and the performance of sub-maximal cycling exercise. It was found that the type and mode of strength training influenced the force generating capacity recovery dynamics post-training with high-intensity and hill cycling strength training sessions causing a greater reduction in MVC torque than low-intensity strength training. Further, that longer duration strength training sessions did not cause any greater reduction in MVC torque than shorter duration training sessions of similar
training intensity. The results also showed that there is an increased physiological cost during subsequent sub-maximal cycling exercise, performed after a period of three hours rest, which was influenced by the type, duration and mode of the prior strength training, in addition to the intensity of the sub-maximal cycling exercise.

To effectively show how the different intensities, durations and modes of training influenced the recovery of force generating capacity and cycling efficiency post-training, comparisons of the protocols corresponding with each of the different training variables have been made. Furthermore, because of the similarity of the findings for Experiments 1A and 1B, the discussions have been combined.

4.6.2. Comparison of Training Intensity (S and SE protocols)

In the study by Behm et al (2002), who compared the effect of a single set of elbow flexion at 5, 10 and 20 RM on the recovery of voluntary and evoked contractile properties, it was found that the 20 RM protocol produced a greater reduction in MVC force and MA as well as a slower recovery rate than the other two protocols. These findings suggest that a lower intensity strength training protocol has a greater effect on force generating capacity and neuromuscular performance than high-intensity protocols. However, the difference may be due to greater volume of work completed for the 20 RM protocol and not the intensity of the protocol per se. It has also been previously reported that the magnitude of the acute decrease in force generating capacity and neuromuscular performance post-strength training is related to the type and overall volume of work as well as the loading intensity of the training session (Komi & Viitasalo, 1977; Kraemer et al., 1991; Kroon & Naeije, 1991). Similarly, it has been reported that the magnitude and duration of EPOC in response to resistance exercise depends on the volume of work, the exercise load and the rest interval between sets (Haltom et al., 1999). In light of the above, the volume of work and the type of loading performed during the warm-up and training sets was equated in the current study as well as the rest intervals between sets. Therefore, the difference between the recovery of force generating capacity and the responses during the efficiency tests post the S and SE training protocols in Experiment 1A may be attributed to the difference in exercise load (intensity) only.

Even though no significant difference was found between the S and SE training protocols in Experiment 1A or 1B of the present study, the reduction in peak and mean MVC torque when
expressed as a percentage of pre-training values, were significant for the S protocol (5.08 and 6.15%; 4.06 and 5.07%) compared to the SE (3.50 and 2.39%; 2.85 and 1.98%), respectively. The trend of a greater reduction for the high-intensity S protocol compared to the low-intensity SE protocol indicates that the intensity of training was probably a factor in the recovery of force generating capacity post-training. To the current investigator’s knowledge, the present study is the first to compare different intensity strength protocols equated for work and highlighted the importance of equating work volume when comparing the recovery of force generating capacity and MA post-exercise. The subjects in Experiment 1A completed 20 repetitions for the SE protocol, the same number of repetitions as the subjects in the study by Behm et al (2002), yet the outcome was the reverse, with a greater reduction in MVC torque for the high-intensity protocol. The difference between the two studies can be attributed to the greater volume of work performed by the prior study for the 20 RM protocol, as the equation of work from a 6 RM load in the current study for 20 repetitions does not equal a 20 RM load but a lower workload. This lower workload reducing the amount of muscle mass recruited during the 20 repetition training set compared to the 20 RM load (Thornton & Potteiger, 2002), thereby reducing the level of fatigue post-exercise.

In view of the greater level of fatigue observed in the present studies, as indicated by the greater reduction in MVC peak and mean torque for the S compared to the SE protocol, it was not surprising to find that the S protocol showed a significantly higher HR response during the loaded workloads of the cycling efficiency test as well as non-significant but consistently higher responses for a number of the physiological variables (RR, TT, BL, GBM, RPE) compared to the SE protocol. However, these differences did not significantly alter cycling efficiency, as similar GE and NE was found for both protocols. The slightly greater elevated physiological responses post the S protocol indicates that a longer recovery period was required for the high-intensity strength training compared to the low-intensity strength training even though the amount of work for the two training sessions was equated. This finding is in accordance with a prior study by Thorton and Potteiger (2002) who showed that a bout of high-intensity strength training resulted in a greater EPOC compared to low-intensity training equal in work volume. Furthermore, the results confirm previous reports that the intensity of strength training is an important factor in determining the magnitude and duration of the recovery period post-exercise (Schuenke et al., 2002).
In the present study the greater level of fatigue post the S compared to the SE training protocol as well as the small difference between the efficiency test responses may be related to a difference in level of muscle damage between the protocols. Even though CK is only considered as an indicator of muscle damage because it is found almost exclusively in muscle tissue (Behm et al., 2001; Newham et al., 1987; Nosaka & Clarkson, 1992), the S protocol (293.5 ± 147.8 U.L⁻¹) did result in a higher CK response compared to the SE protocol (197.0 ± 66.9 U.L⁻¹), a finding that is in accordance with previous literature reports that high-intensity strength training results in a greater CK response compared to low-intensity training (Tiidus & Ianuzzo, 1983). The differences in the recovery of force generating capacity and the efficiency test results may also be associated with the degree of disturbance of pre-exercise state as indicated by the significantly higher BL response post the S training protocol (4.85 ± 1.79 mmol.L⁻¹) compared to the SE protocol (2.41 ± 1.02 mmol.L⁻¹) in Experiment 1A. The higher BL response for the S protocol indicating a greater dependency on anaerobic metabolism via increased contribution of glycolytic muscle fibres compared to the SE protocol and is in accordance with previous research that has shown a relationship between exercise intensity and the type of muscle fibres recruited for exercise (Gollnick et al., 1974; Sale, 1987; Takaishi, Yasuda, Ono, & Moritani, 1996).

4.6.3. Comparison of Training Duration (S and SUL protocols)

It has previously been suggested that muscle activation and force production following fatiguing sub-maximal strength exercise are duration dependent and decrease to a greater degree as the duration of the session increases (Behm & St-Pierre, 1997). However, the current findings indicate that the influence of exercise duration on the recovery of force generating capacity and MA does not hold true for strength training where the duration of the sessions is increased by the inclusion of additional exercises that do not use the same muscle groups as those used for prior exercises completed in the same session. The similar significant reduction in MVC mean torque for the SUL and S protocols in Experiment 1B as well as no change in MA suggests that the duration of the training session via the inclusion of two additional exercises was not a factor in the recovery of force generating capacity and MA post-training. This finding and the fact that the same leg-exercise and intensity was completed for both protocols suggests that the recovery of force generating capacity and MA following the training sessions was localised to the actual muscles involved in the leg-exercise and not to a systemic effect that may have come from the inclusion of upper body exercises. However,
the similar reductions in MVC torque for the S and SUL protocols does not mean that a systemic effect of the SUL training did not occur, as the MVC and twitch interpolation techniques do not test whole body function.

It is also possible that the similarity between the SUL and S protocols was a consequence of the order of the exercises in the SUL protocol. In an effort to explain why there was no significant reduction in MA immediately following a series of strength exercises commencing with two high-intensity exercises followed by four lower intensity exercises, Häkkinen (1990) outlined that the exercise order may have provided more time for the previous exercised muscles to recover prior to testing. Whilst this is a possible explanation for the current finding due to the leg-press being completed first in the SUL protocol, it is unlikely to have caused a significant effect given that testing was performed three hours post-training, unlike Häkkinen (1990) who tested immediately following training. Therefore, the contribution of the extra 40 minutes of recovery to the result would have been reduced due to the three hours duration between the finish of the training sessions and the MVC tests.

Prior investigations of endurance exercise have shown that duration of exercise, in combination with intensity, are important factors in determining the rate of post-exercise recovery when assessed by EPOC (Bahr et al., 1987; Gore & Withers, 1990; Mæhlum et al., 1986). Comparison of the efficiency test after the S and SUL protocols supports this comment, as the SUL protocol produced higher physiological responses across the majority of the variables compared to the S protocol as well as a small reduction in GE and NE. Even though the difference between the S and SUL protocols was not significant, the findings are in line with previous reports that have shown that longer duration endurance exercise protocols have a greater effect on recovery dynamics than shorter exercise protocols of similar intensity (Bahr et al., 1987; Quinn et al., 1994; Sedlock et al., 1989). The higher physiological responses for the SUL protocol compared to the S protocol suggests that unlike the MVC torque results, where there appeared to be a similar localised effect of the prior strength training bouts, the efficiency test results indicate more of a systemic effect. The greater disturbance of exercising responses following the SUL protocol compared to the S protocol may have been due to a greater volume of exercising muscle mass during the training session due to the inclusion of two additional exercises (bench press and lat pull-down), a suggestion that has been proposed previously to explain differences in EPOC post-different types of training (Elliot et al., 1992). The significantly higher BL concentration post the SUL protocol
compared to the S protocol supports this line of reasoning. The greater physiological response during the efficiency test following the SUL protocol also indicates that a longer prior training duration can create a greater and longer lasting physiological stress than a shorter duration protocol of similar intensity.

4.6.4. Comparison of Training Modes (H, S, SE and SUL protocols)

In a recent review of fatigue during high-intensity intermittent exercise, Lambert and Flynn (2002) outlined that the extrapolation of data from other modes of training (e.g. 30 second all-out cycle tests) to resistance training was limited because the level of fatigue and amount of work performed was different even if performed for a similar duration as resistance training. However, a comparison can be made if the amount of work is equated (Thornton & Potteiger, 2002) and the speed of the movement similar (H protocol = 17 revolutions, SE protocol = 20 repetitions), as was used in Experiments 1A and 1B of the present study. The S and SE protocols, were equated in terms of the amount of work completed for the six training sets, whilst for the H protocol twice the volume of work was completed to avoid using unrealistic workloads or very short repetition intervals.

To date, the comparison of strength training modalities such as hill cycling and conventional strength training on the recovery of force generating capacity or strength development is not available. Therefore it is difficult to compare the effects of short periods (20 seconds) of slow hill cycling, which involves only concentric contractions (Knuttgen, 1986), with similar duration conventional weight training sets incorporating both concentric/eccentric contractions (Byrnes, Clarkson, & Katch, 1985; Jamurtas et al., 2000). However, studies that have compared eccentric and concentric only contractions/exercises have reported that there is a greater decrease in muscle function following the eccentric contractions than the concentric contractions (Ebbeling & Clarkson, 1989; Komi & Viitasalo, 1977; Kroon & Naeije, 1991; Sargeant & Dolan, 1987). In light of this, it was anticipated that the H protocol of the present study would show minimal change in MVC torque post-exercise. However, this was not found, as the H protocol produced a reduction in peak and mean torque nearly equal to that of the S and SUL protocols. The similarity between the H and high-intensity strength protocols does not appear to be due to fatigue resulting from muscle damage, as indicated by the difference in the CK response. The S protocol showed the greatest CK response from pre- to post-training, increasing by 74% compared to the SE and H protocols, which increased or
decreased marginally by 22% and 2%, respectively. However, these results do confirm previous reports that exercises involving eccentric work components produce greater CK responses than exercises involving only concentric components (Clarkson, 1997; Evans & Cannon, 1991).

In the current study, the similar decrease in MVC peak and mean torque for the S and H protocols is contrary to previous reports of greater decrements in neuromuscular performance following exercise with eccentric contractions compared with exercise with only concentric contractions (Ebbeling & Clarkson, 1989; Komi & Viitasalo, 1977; Kroon & Naeije, 1991; Newham, McPhail et al., 1983; Sargeant & Dolan, 1987). It is possible that the reduction in peak and mean torque for the H protocol similar to that of the S protocol may have been due to the greater amount of work performed for the H protocol or the high-level of tension generated within the muscle compared to the other protocols (Giddings, Neaves, & Gonyea, 1985). These findings may have resulted in a higher level of muscle fatigue than otherwise would have been occurred if an equal amount of work were completed. Whilst the reason for the above findings is not clear and requires further research, it does suggest that the type of fatigue following cycling exercise may be different to that found for resistance training (Gibala et al., 1995).

A comparison of the efficiency tests following the strength training protocols supports the above notion of a difference in the type of fatigue post-exercise as a slightly greater physiological cost, reflected by elevated responses in a number of variables (HR, RR, V̇E, BL, TT and GBM), was found for the S and SUL protocols compared to the H protocol. A greater reduction in GE and NE, though not significant, was also found for the S and SUL protocols compared to the H protocol. These findings are consistent with a prior study by Gleeson et al (1995) who compared 30 minutes of bench stepping and up-hill walking and its effects on the physiological responses during a bout of sub-maximal cycling performed two days later. They reported that exercise with a large eccentric component produced physiological responses indicative of a higher relative exercise stress than exercise with concentric contractions only. Gleeson et al (1995) also found that there was no increase in serum CK following the up-hill walking exercise bout, similar to that of the current findings.

Comparison of the S, SE and H efficiency tests in the present study also showed that the volume of work completed during the prior training sessions was not a major contributing
factor to the overall result. If so, then the efficiency test post the H protocol should have shown greater levels of physiological stress than both the S and SE protocols but this was not the case. Again this suggests that fatigue for cycling exercise may be different to that found for conventional strength training exercise. This suggests that a greater amount of work can be completed using the H protocol than conventional strength training before a similar level of muscle fatigue is experienced. Furthermore, that the interference of fatigue following the H protocol on sub-maximal cycling performance is less than that of conventional high-intensity strength training. Further investigation is required into the factors contributing the relationship between fatigue following the H protocol and the MVC results and why a similar level of physiological cost as the S and SUL protocols was not found during the efficiency test in light of the similar reductions in peak and mean torque. However, the findings do suggest that hill cycling may be more beneficial for cyclists due to less interference in sub-maximal cycling exercise performed post-exercise than conventional strength training, an issue that is addressed in more detail in the Practical Implications (Section 8.7) in the Summary and Conclusion, Chapter 8.

4.6.5. Common Findings

Following the comparison of the MVC trails and efficiency tests after the various training protocols, three common findings were observed.

4.6.5.1. The contribution of peripheral fatigue to the reduction in maximal voluntary contraction torque

Reductions in force generating capacity following high-intensity strength training have been shown to be associated with decreases in muscle activation (Häkkinen et al., 1988). The voluntary MA results in Study 1 do not seem to support this notion, as no significant difference was found between the strength training protocols or from pre- to post-training and as such suggests that changes in voluntary MA were not responsible for the reduction in force generating capacity. However, the voluntary MA responses disguise the fact that there was a significant reduction in post-stimulation peak torque (superimposed twitch), which paralleled a reduction in MVC torque. The combination of the MVC peak torque plus that proportion achieved above this level after the superimposed twitch stimulus provides an indication of the total amount of torque that can be generated by a muscle (Enoka, 2002). The results for
Experiment 1A showed that the total amount of torque generating capacity available, as reflected by the superimposed twitch peak torque (data not shown), decreased significantly (indicated by an asterisk, *) for two of the three protocols (S* 4.87; SE 2.96 and H* 4.11%) and in similar proportion to the reduction in MVC torque (S 6.15, SE 2.39 and H 4.22%) when the reductions in torque are expressed as a percentage of pre-training values. Experiment 1B also showed a similar proportional decrease in superimposed twitch peak torque (SUL* 6.17, S 4.87, SE 2.07 and H* 5.24%) compared to the reduction in MVC torque (SUL 5.16, S 5.38, SE 1.31 and H 5.70%). Such similar reductions in the superimposed twitch and MVC torque have also been reported previously (Bigland-Ritchie et al., 1986; Jones, 1996).

In conjunction with the above, a reduction in the control twitch for all of the training protocols was also found. The reduction in the control twitch when expressed as percentage of pre-training values, showed that there was a greater reduction for the S protocol compared to the other two training protocols in Experiment 1A. A similar reduction in the control twitch response for the SUL protocol to that of the S protocol was also found for Experiment 1B. The reduction in the control twitch responses along with those of the superimposed twitch responses and MVC torque, indicate that peripheral muscle fatigue was the major contributor to the reduction in MVC torque following all the strength training protocols (Bigland-Ritchie et al., 1986; Enoka, 2002).

4.6.5.2. Increased physiological cost during the efficiency tests following the strength training protocols

All the efficiency tests following the strength training protocols displayed elevated physiological responses during both the rest and exercise periods to varying levels depending on the type, duration and mode of training compared to the control test in both Experiments 1A and 1B. These findings suggest that the completion of strength training in the hours prior to endurance exercise can increase the physiological cost of exercise. Further, that a greater period of time between the strength training sessions and efficiency tests is required to bring the physiological processes back to equilibrium (Thornton & Potteiger, 2002). The results of both current experiments also revealed that a number of the variables (HR, TT, BL and RPE) measured during the efficiency tests following the S and SUL protocols showed sensitivity to the exercise intensity by increasing at a greater rate and magnitude than for the other
efficiency tests following the SE and H protocols. This sensitivity to high workloads may be related to the level of reduction in MVC torque and the greater CK response found for these two protocols requiring the recruitment of additional motor units to maintain power output and hence an increase in energy expenditure (Seabury, Adams, & Ramey, 1977).

4.6.5.3. The influence of training age and/or VO$_2$ max of the subjects on recovery post-training

The present data suggest that the level of recovery following the strength training sessions may have been influenced by the training age and/or VO$_2$ max of the subjects. The subjects were regularly undertaking strength and endurance training prior to the study and as such may have adapted to the stress of regular training more than lesser trained or untrained subjects (Melby et al., 1992; Thornton & Potteiger, 2002). Evidence that this may have been the case was provided in the observation from the MVC trails and efficiency test results that those subjects with a greater training age and/or VO$_2$ max showed lower reductions in peak and mean torque and reduced levels of physiological stress than those of a lesser training age and/or VO$_2$ max, respectively.

In addition to the mechanisms contributing to the reduction in force generating capacity and the increased physiological cost and changes in GE and NE during the efficiency tests, the abovementioned issues will also be addressed in more detail in the General Discussion.

4.6.6. Influence of training volume on recovery capacity post-exercise

Apart from the possible influence of the duration of the recovery between the strength training sessions and the efficiency tests, the lack of a significant difference between the recovery of force generating capacity and the efficiency test results for the protocols in Experiment 1A and 1B of the present study may have been due to the volume of strength training performed in the respective strength training sessions. Previous strength training studies that have shown significant reductions in force generating capacity or neural properties for extended periods of time post-training (hours to days) have used high training volumes with as many as 20 sets of varying intensity (1-10 RM) (Gibala et al., 1995; Häkkinen, 1993; Linnamo et al., 1998) whilst those studies that have used single sets of varying intensity (5-20 RM) (Behm, Reardon et al., 2002) have shown non-significant and notably shorter recovery periods.
Similarly, previous strength training studies (Dolezal et al., 2000; Gillette et al., 1994; Schuenke et al., 2002) that have found that metabolism and oxygen consumption post-exercise remains elevated for extended periods of time (> 12 hours) have utilised protocols consisting of eight to ten exercises for four or more sets, with each set to failure using a load that could be lifted for 8-12 repetitions (Schuenke et al., 2002). In contrast, those studies that have not shown a lasting effect (< two hours) have used either very low resistances such as 50% of 1RM loads (Elliot et al., 1992; Haltom et al., 1999; Murphy & Schwarzkopf, 1992) or high-intensity loads that could only be moved 3-6 repetitions (Elliot et al., 1992), an observation reported by Schuenke and colleagues in a recent study (2002). Therefore, given the low- and high-intensity loads and long rest periods used in the protocols of Experiments 1A and 1B of the present study, it is possible that this may have produced a lower level of stress than greater volume and moderate intensity-protocols used previously (Kraemer, Noble, Clark, & Culver, 1987; Thornton & Potteiger, 2002; Wilson, 1994b). The current findings along with those of previous studies as outlined above, indicates that strength training protocols with greater training volumes like the SUL protocol or those aimed at producing hypertrophy cause a greater magnitude and longer lasting disturbance of pre-exercise state than shorter duration sessions of low- or high-intensity (Schuenke et al., 2002). A factor that has implications for concurrent training programmes where high strength training volumes are used or where hypertrophy is the main focus of the strength training component as well as the scheduling of strength and endurance sessions within the one day.

4.6.7. Summary and Conclusion

In summary,

- There was a greater reduction in peak and mean MVC torque for the S protocol compared to SE protocol when equated for work, which may be attributed to some form of peripheral fatigue, possibly stemming from muscle damage. Further, the H protocol, which involved only concentric contractions but twice as much work produced similar reductions in peak and mean MVC torque as the S and SUL protocols, which involved eccentric contractions.
The longer duration SUL protocol, which incorporated exercises for both upper and lower body muscle groups, produced a localised effect on the recovery of force generating capacity. In contrast, the increased physiological cost and subsequent reduction in GE and NE during the efficiency tests post the SUL protocol seemed to be due to a systemic effect.

The differences between the efficiency tests post the strength training protocols appear to have been related to the intensity and duration of the prior exercise as well as the mode of training, with the SUL and S training protocols exhibiting higher physiological stress than the SE and H protocols. In addition, the effect of the training protocols on subsequent cycling exercise was workload sensitive, with higher workloads producing a greater physiological response than lower workloads.

The level of muscle damage incurred by subjects, as reflected by changes in CK activity, was greater for the S and SUL protocols than the SE or H protocols. Furthermore, the mechanisms contributing to the reduction in force generating capacity and increased physiological cost of cycling post-training appeared to be different for the S and SUL protocols compared to the H protocol.

It was concluded that these findings support the hypotheses outlined in Chapter 1. First, that the high-intensity S protocol had a greater residual physiological effect on muscle force generating capacity and the physiological cost of cycling than the low-intensity SE protocol when equated for work volume. Second, that the 60-minute high-intensity SUL protocol had a greater residual physiological effect than the 30-minute high-intensity S protocol, as well as conventional strength training incorporating both eccentric and concentric contractions compared to the H protocol, which utilised only concentric contractions. In light of these findings, the intensity, duration and mode of strength training must be considered when designing a concurrent training regime in order to minimise the residual physiological effect on subsequent endurance exercise performed on the same day.
CHAPTER 5

Study 2

The acute effect of the sequence of strength and endurance training on muscle force generating capacity and cycling performance, post-training

5.1. Introduction

Investigations of the compatibility of concurrent training on strength and endurance adaptation have used a variety of training combinations including endurance training prior to strength training (Kraemer et al., 1995; Sale et al., 1990a) and strength training prior to endurance training (Hickson et al., 1988; Hortobágyi et al., 1991; Sale et al., 1990a) as well as the scheduling of the training sessions including same day (Craig et al., 1991; Dolezal & Potteiger, 1998; Sale et al., 1990b) and alternate days (Bell et al., 2000; Dudley & Djamil, 1985; Sale et al., 1990b). However, only a limited number of studies (Bell et al., 1988; Bell, Petersen, Wessel, Bagnall, & Quinney, 1991a; Collins & Snow, 1993; Gravelle & Blessing, 2000) have directly investigated the sequence of training within the one training regime on strength and endurance adaptations. It remains unclear from the findings of these investigations, due to contrasting results and differences in the types of training used, as to the acute effect that the sequence and frequency of training have on strength and endurance responses.

The aim of Study 2 was to examine the acute residual physiological effects of two sequences of strength and endurance training completed on the same day on muscle force generating capacity and cycling efficiency three hours post-training and blood variables three and 24 hours post-training. Further, to examine whether the sequence of completing strength and endurance training sessions effected,
• the physiological stress of the second training session compared to when the training sessions were completed first, and

• the recovery dynamics of both muscle force generating capacity and physiological parameters post the training sessions.

5.2. Methods

5.2.1. Study Design

After completing a familiarisation session, as described in the General Methodology and Materials Chapter (Section 3.2.2), each subject was tested on four separate occasions over a four-week period. The first two test occasions were control sessions (VO₂ max test and a ‘control day’) followed by two different training sequences (termed ‘sequence days’) over a two-week period, incorporating weight (W) and endurance cycle (C) training.

On each of the sequence days the subjects arrived at the laboratory 30 minutes prior to the first training session and rested before completing an isometric strength/muscle activation test and a one-hour W or C training session, as described under the heading ‘Training Protocols’ below. Three hours after the completion of the first training session the subjects completed a second isometric strength/muscle activation test and training session (C or W, respectively). Two and a half hours after the completion of the second training session the subjects returned to the laboratory and rested for 30 minutes before completing another isometric strength/muscle activation test and cycling efficiency test. Twenty-four hours after the pre-W training time point, the subjects returned to the laboratory to provide a blood specimen (Figure 5.1.). The trial days commenced between 06:00 and 08:00 hours. Whilst there was some variation in the start time between subjects, there was no within-subject variation.

The control days were a duplicate of the sequence days (Figure 5.1) except that no actual W or C training sessions were completed in the respective training time slots. Further, no 24-hour post W training session blood specimen was collected.

Throughout the duration of the control and sequence days, the subjects were permitted to leave the laboratory to attend University classes and study however they were not permitted to participate in any physical activity. Dietary intake was controlled throughout each of the trial
days as described in the ‘Subject Preparation’ (Section 3.2) in the General Methodology and Materials Chapter. The subjects were randomly allocated to one of the two training sequences, with four subjects completing each sequence (WC = weight then endurance cycle training, CW = endurance cycle then weight training).

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 1.</td>
<td>WC</td>
<td>CW</td>
</tr>
<tr>
<td>Sequence 2.</td>
<td>CW</td>
<td>WC</td>
</tr>
</tbody>
</table>

There was a minimum of three days between the familiarisation session and VO2 max test and seven days between the VO2 max test, control day and each of the sequence training days, in order to eliminate any possible carry over effects from one test to another.

5.2.2. Subjects

Eight (8) male athletes who were actively participating in cycling and weight training on a regular basis, volunteered for the study. Of the eight subjects, four were from Study 1 (Experiment 1B). Their mean (±SD) age, height, body mass and VO2 max (absolute and relative) are outlined in Table 5.1.

The subjects were selected according to the following criteria.

- Aged between 18 and 40
- Currently cycling a minimum of 2-3 times per week with an approximate weekly duration total of 2-3 hours
- A minimum of 12 months cycling and weight training experience
- Currently performing lower and upper body resistance exercises a minimum of two times per week
- No contraindications to the test procedures
Table 5.1. Descriptive characteristics of the eight subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>(±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.79</td>
<td>0.07</td>
</tr>
<tr>
<td>Body Mass (kilograms)</td>
<td>75.05</td>
<td>6.44</td>
</tr>
<tr>
<td>VO$_2$ max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (L.min$^{-1}$)</td>
<td>4.31</td>
<td>0.71</td>
</tr>
<tr>
<td>Relative (mL.kg$^{-1}$.min$^{-1}$)</td>
<td>57.51</td>
<td>9.03</td>
</tr>
</tbody>
</table>
Training and Muscle Activation Schedule

Control Day

Sequence Day (WC or CW)

Blood Sampling Schedule

Figure 5.1. Study Design: An example of the control day and sequence days’ time lines.

The dark shaded areas refer to the first (W or C) and the second (C or W) training sessions, respectively whilst the light shaded area refers to the cycle efficiency test. The large and small arrows reflect the muscle activation and blood sampling time points, respectively. The small light coloured arrows for the “blood sampling schedule” refer to those time points that were 24 hours after the pre W specimen (07:00 hours for the WC and 11:00 hours for the CW, respectively). The small long arrows refer to those sampling time points applicable to the control day only.
5.2.3. Training Protocols

Subjects completed the following training protocols for both sequence day 1 and 2.

Weight (W) Training

Warm-up: Five minutes of stationary cycling at a relative workload corresponding to 1 watt per kg of GBM followed by two sub-maximal trials at 30% and 60% of leg- and bench-press 1 RM and lat pull-down 6 RM for 10 and five repetitions, respectively.

Training Sets:
- Leg-press 6 x 6 RM
- Bench-press 4 x 6 RM
- Lat pull-down 4 x 6 RM

The 6 RM loads were calculated as 85% of each subject’s bench- and leg-press 1 RM (Feigenbaum & Pollock, 1997; Hoeger et al., 1990) and 100% of each subject’s lat pull-down 6 RM.

Rest Intervals: One-minute between warm-up trials and three minutes between training sets

Duration: ~60 minutes

Cycle Endurance (C) Training

Warm-up: Five minutes of level cycling at a workload corresponding to 40% of VO₂ max

Cadence: 90 rpm

Intensity: Workload corresponding to 60% of VO₂ max

Duration: 60 minutes
5.2.4. Sampling Protocols and Measurements

In addition to the sampling time points and variables measured for the isometric strength/muscle activation tests, strength training sessions and cycling efficiency tests as described in Study 1, blood specimens and variables were also collected or measured prior to, during and following the C training session, as outlined below. The specimens and variables were collected and analysed as described in the corresponding section in the General Methodology and Materials (Chapter 3).

5.2.4.1. Isometric strength/muscle activation (twitch interpolation)

Three MVC trials along with three voluntary and involuntary MA trials using the twitch interpolation technique, as described in the General Methodology and Materials Chapter (Sections 3.3.2. and 3.3.3, respectively), were completed 10 minutes prior to the endurance cycle training sessions. The completion of the same MVC and MA trials before the C training sessions, as used for the W training sessions and control day, enabled the subject’s muscle force generating capacity pre and post the different training sessions to be compared.

5.2.4.2. Blood Specimen Collection

Capillary blood specimens were collected pre and immediately after each cycling endurance training session for the determination of pre- and post-exercise blood lactate concentration. The post-exercise specimens were collected within 60 seconds of completing each training session and before dismounting the cycle ergometer.

Venous blood specimens were collected at the following time points:

- immediately prior to each day’s initial training session, and
- prior to the isometric strength/muscle activation test and subsequent cycling efficiency test at the end of each control/sequence day,

for the determination of resting,

- serum testosterone and cortisol concentrations.
For the venous blood specimens, the subjects reported to the laboratory 30 minutes prior to the respective test/training session and were laid supine on a bed in a quiet air-conditioned room to rest before the blood specimen was collected. Two subjects failed to have venous blood specimens collected, one due to technical difficulties and the other preferring not to have blood collected.

5.2.4.3. Respiratory Gases

Expired respiratory gases were collected during the five-minute rest and warm-up intervals and throughout the C training sessions, over every second 10-minute interval (i.e. at 11-20, 31-40 and 51-60 minutes). The measuring of respiratory gases in intervals allowed for fluid intake at regular intervals throughout the training session. A standardised volume of water (3 x 200 mL = 600 mL total) was provided for all training sessions. In addition to reporting the last five minutes of each 10-minute period for the respiratory variables, VO₂, Vₑ and RR, the respiratory gases were used to estimate energy expenditure for the respective sampling periods. The energy expenditure estimates were then used to calculate GE and NE as described in the General Methodology and Materials Chapter (Section 3.5.8).

5.2.4.4. Heart Rate

Heart rate and ECG were monitored continuously throughout the C training sessions and reported for the same time periods as used for the expired respiratory gases.

5.2.4.5. Body Temperature Monitoring

Tympanic temperature was measured immediately prior to and post the C training sessions.

5.2.4.6. Gross-Body-Mass

The subject’s GBM was measured prior to and immediately post the C training sessions.

5.2.4.9. Rating of Perceived Exertion

The RPE scale was also used to monitor each subject’s perception of effort during the C training sessions and recorded during the last 15 seconds at the end of every second 10-minute interval.
5.2.5. Statistical Analysis

Descriptive statistics (mean and SD) were calculated for all test and performance variables.

5.2.5.1. Comparison of the Weight and Endurance Cycle Training Variables

A three-factor repeated measures ANOVA with two-within subject factors, training sequence (weights-cycle, WC and cycle-weights, CW) and time (pre- and post-training) and one-between subject factor, test order was completed in order to determine those training variables that responded differently to the training sequences. This analysis was completed for the weight and/or endurance cycle training dependent variables BL concentration, CK, GBM and TT.

A three-factor repeated measures ANOVA with two-within subject factors, training sequence (WC and CW) and time (rest; warm-up, 11-20, 31-40 and 51-60 minute periods) and one-between subject factor, test order was completed in order to determine those endurance cycle training variables that responded differently to the training sequences. This analysis was completed for the dependent variables HR, RR, $V_E$, $VO_2$, GE, NE and RPE.

5.2.5.2. Comparison of the Maximal Voluntary Contraction and Muscle Activation Responses for the Control Day and Sequence Days

A three-factor repeated measures ANOVA with two-within subject factors, training sequence (WC and CW) and time (pre the first and second training sessions and pre-efficiency test) and one-between subject factor, test order was completed in order to determine those variables that responded differently to the training sequences. This analysis was completed for the dependent variables peak and mean torque and MA.

5.2.5.3. Comparison of the Efficiency Test Variables for the Control Day and Sequence Days

A three-factor repeated measures ANOVA with two-within subject factors, trial day (WC, CW and control) and period and one-between subject factor, test order was used to determine if there was a difference between the control day and training sequences for all efficiency test variables. For those variables with more than four data points for the within subject factor
period, it was necessary to split the analysis into two: (one) rest and unloaded cycling, (two) 20, 40 and 60% of VO$_2$ max workload periods. This analysis was completed for the dependent variables HR, RR, $V_E$, VO$_2$, TT and BL concentration. A single three-factor (trial day by period by test order) repeated measures ANOVA was completed for the variables GE, NE, GBM and RPE where there were a reduced number of periods.

5.2.5.4. Comparison of the Hormone Responses for the Control Day and Sequence Days

A three-factor repeated measures ANOVA with two-within subject factors, trial day (WC, CW and control) and time (pre- and post-training) and one-between subject factor, test order was used to determine if there was a difference between the control day and training sequence days for testosterone, cortisol and testosterone/cortisol ratio.

In the event of a violation of the sphericity assumption for any of the dependent variables, the Greenhouse-Geisser correction was used. For all analyses that indicated a significant effect, Bonferroni adjusted multiple pair-wise comparisons were completed to locate the source of the difference. An alpha level of .05 was used to indicate a level of significant difference for all ANOVA analysis. All statistical analysis was completed using the Statistical Package for Social Sciences (SPSS version 11.0 for windows, SPSS Inc.).
5.3. Results

5.3.1. Effect of Test Order

The between-subjects factor (test order) was included in all variable statistical analysis undertaken. No effect of test order was found for any of the weight training, endurance cycle training, MVC, MA or efficiency test variables measured (p>.05). To avoid unnecessary repetition of this finding within each variable result, a list of the variables and the corresponding statistical result are provided in Appendix F.

5.3.2. Comparison of the Weight Training Variables

5.3.2.1. Blood Lactate Concentration

A significant main effect of training sequence (p=.012) and time (p<.001) was found however, there was no interaction of training sequence by time (p=.168). Post-hoc analysis showed that there was a significantly (p=.032) higher pre W and near significant (p=.056) post W BL concentration for the CW sequence (1.15 ± 0.30 and 6.61 ± 1.81 mmol.L⁻¹) compared to the WC sequence (0.83 ± 0.20 and 5.51 ± 1.42 mmol.L⁻¹), respectively (Figure 5.2, top). Post-hoc analysis indicated that both training sequences showed significant increases (p<.001) in BL concentration compared to pre-training values.

5.3.2.2. Creatine Kinase Activity

No significant difference was found between the training sequences (p=.247) or interaction of training sequence by time (p=.132) (Figure 5.2, bottom). There was however, a significant main effect of time (p=.018). Post-hoc analysis indicated that compared to pre CK activity (CW 118.5 ± 39.6 U.L⁻¹, WC 189.8 ± 72.1 U.L⁻¹), a significant increase in post-training activity was found in respect to the CW sequence (p=.026, 209.5 ± 90.4 U.L⁻¹) but not the WC sequence (p=.249, 222.6 ± 77.9 U.L⁻¹).
**Figure 5.2.** Pre- and post-training BL concentration (top) and CK activity (bottom) for the weight training session of the WC and CW sequences (Mean, error bars represent SD).

* : Significantly greater than pre (p<.05)
# : Significantly greater than WC (p<.05)
5.3.3. Comparison of the Endurance Cycle Training Variables

5.3.3.1. Blood Lactate Concentration

A significant main effect of training sequence (p=0.036) and time (p<0.001) was found, as well as an interaction of training sequence by time (p=0.019). Post-hoc analysis showed that there was a significant (p<0.001) increase in post-training BL concentration compared to pre-training for both training sequences (Figure 5.3). Whilst no difference was found between the WC (1.04 ± 0.39 mmol.L⁻¹) and CW (0.87 ± 0.20 mmol.L⁻¹) pre-training concentrations, the post-training BL concentration for the WC sequence (2.41 ± 0.48 mmol.L⁻¹) was significantly (p=0.004) greater than the CW sequence (1.85 ± 0.22 mmol.L⁻¹).

![Figure 5.3. Pre- and post-training BL concentration for the endurance cycling training session of the WC and CW sequences (Mean, error bars represent SD).](image)

* : Significantly greater than pre (p<.001)
# : Significantly greater than CW (p<.05)
5.3.3.2. Heart Rate

A significant main effect of sequence (p=.001) and time (p<.001) was found. There was also a significant interaction of training sequence by time (p=.009). Post-hoc analysis of the HR response at each of the time intervals throughout the cycling training session indicated a significantly greater response for the WC sequence at the time intervals rest (p=.003, 16.3%) and warm-up (p=.002, 6.10%) compared to the CW sequence, respectively (Figure 5.4). No significant (p>.05) difference was found between the remaining time points over the duration of the cycling session as the difference between the sequences reduced to approximately 2% by the end of the session. Given the linear relationship between HR and workload at sub-maximal levels, HR increased significantly (p<.001) from rest to warm-up through to the 11-20 minute interval for both training sequences. However, over the duration of the 60 minute cycling session there was a small increase in the HR response from one sampling time interval to the next for both sequences but the increases were not significant (p>.05).

Figure 5.4. Heart rate response for the WC and CW training sequences during the rest and warm-up periods and the 11-20, 31-40 and 51-60 minute time periods of the endurance cycle training session (Mean, error bars represent SD).

* : Significantly greater than CW (p<.05)
5.3.3.3. Respiratory Responses

No significant difference was found between the training sequences other than for RR (p=.011), even though the average increase in RR, $V_e$ and $V_O_2$ for the WC training sequence above that of the CW sequence over the duration of the cycle sessions was 5.80%, 9.64% and 9.12%, respectively (Figure 5.5a). Ventilation did approach significance (p=.074). Post-hoc analysis of the RR response revealed a significant difference between the training sequences for the three time periods 11-20, 31-40 and 51-60 minute (p=.018, p=.031 and p=.020, respectively). No significant difference was also found between the training sequences for RER (p=.219), with a maximum increase of 4% for the WC sequence above that of the CW sequence over the duration of the cycle session (Figure 5.5b).

A significant main effect of time was found for the respiratory variables RR, $V_e$ and $V_O_2$ as well as RER (p<.05). Post-hoc analysis revealed that there was a significant increase in RR, $V_e$ and $V_O_2$ from rest to warm up, warm up to the 11-20 minute time period and from the beginning of the 60 minute cycling session to the end of the session (p<.05) for the WC training sequence. The respiratory responses RR, $V_e$ and $V_O_2$, all increased significantly (p<.05) from rest to warm-up and from warm-up to the 11-20 minute time period for the CW sequence. However, only RR increased significantly (p<.05) from the beginning to the end the cycling session. Following a significant increase in RER from the warm-up to 11-20 minute time period both the WC and CW sequence showed a significant reduction in RER over the duration of the cycle session (p<.05).

There was no interaction of training sequence by time for any of the respiratory response variables (p>.05).
Figure 5.5a. Respiratory responses, RR (top), $V_E$ (middle) and $VO_2$ (bottom) during the rest and warm-up periods and the 11-20, 31-40 and 51-60 minute time periods of the endurance cycle training session for the WC and CW training sequences (Mean, error bars represent SD).

*: Significantly greater than CW ($p<.05$)
Figure 5.5b. Respiratory exchange ratio (RER) during the rest and warm-up periods and the 11-20, 31-40 and 51-60 minute time periods of the endurance cycle training session for the WC and CW training sequences (Mean, error bars represent SD).

* : Significantly greater than warm-up period (p<.05)
# : Significantly less than 11-20 and 31-40 min period (p<.05)
5.3.3.4. Gross and Net Efficiency

There was no significant difference between the two training sequences for GE (p=.134) and NE (p=.430) during the warm-up period or the 11-20, 31-40 and 51-60 minute time periods of the cycle session (Figure 5.6, top and bottom, respectively). A significant main effect of time was found for GE only (p<.001). Post-hoc analysis indicated that GE increased significantly (p=.013) for the WC sequence from the warm-up to the 11-20 minute time period but then significantly (p=.018 and p=.029) decreased at the 31-40 and 51-60 minute time periods, respectively. There was no significant change over the duration of the CW sequence cycling session. No interaction of training sequence by time was found for either GE (p=.247) or NE (p=.356).

5.3.3.5. Tympanic Temperature

A significant main effect of training sequence (p=.002) was found (Table 5.2). Post-hoc analysis revealed a significantly higher pre- (p=.002) and post-training (p=.002) TT for the cycling session following the weight training session compared to when the cycling session was completed prior to the weight training session. Whilst there was no main effect of time found, it did approach significance (p=.060), suggesting a notable increase in TT over the duration of the cycling session. No interaction of training sequence by time (p=.162) was found.

5.3.3.6. Gross-Body-Mass

The same statistical and post-hoc analysis, as used for TT above, was also used for GBM. All subjects, in both the WC and CW sequences, experienced small but significant (p<.001 and p.004, respectively) reductions in GBM following the cycling training session when compared to pre-training values (Table 5.2). Even though subjects experienced a greater GBM reduction for the WC sequence (0.74%) compared to the CW sequence (0.54%), the difference was not significant (p=.649). A significant interaction of training sequence by time (p=.002) was also found, indicating that the influence of the sequence of training on GBM reduction was dependent upon the time of the measurement.
Figure 5.6. Gross (top) and net (bottom) efficiency during the warm-up period and the 11-20, 31-40 and 51-60 minute time periods of the endurance cycle training session for the WC and CW training sequences (Mean, error bars represent SD).

# : WC sequence, significantly different to warm-up (p<.05)
* : WC sequence, significantly different to 11-20 min period (p<.05)
Table 5.2. Tympanic temperature and GBM pre and post the endurance cycling training session for the WC and CW training sequences (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th></th>
<th>WC</th>
<th></th>
<th></th>
<th>CW</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tympanic Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.2*</td>
<td>37.6*</td>
<td>36.9</td>
<td>37.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.2)</td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBM (kg)</td>
<td>75.73</td>
<td>75.17#</td>
<td>75.73</td>
<td>75.33#</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.43)</td>
<td>(6.47)</td>
<td>(6.51)</td>
<td>(6.33)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* : Significantly greater than CW (p<.05)
# : Significantly less than pre (p<.05)

Note: GBM post-training values include the standardised 600 mL of water consumed during the endurance cycling training session.
5.3.3.7. Rating of Perceived Exertion

Even though subjects indicated a higher RPE for the WC sequence at the 20, 40 and 60 minute time periods during the cycling training session (Figure 5.7), there was no significant difference between the training sequences (p=.320) or interaction of training sequence by time (p=.085). However, a significant (p<.001) main effect of time was found indicating that the timing of the measurement influenced the RPE response. Post-hoc analysis for all the time points across the two sequences revealed that the subjects experienced a significantly (p<.05) greater RPE at each time point for the WC sequence than the time period before it other than the 51-60 minute time period. In contrast, no significant difference was found between any of the time points for the CW sequence other than the warm-up and 11-20 minute time periods.

Figure 5.7. Ratings of perceived exertion during the warm-up period and the 11-20, 31-40 and 51-60 minute time periods of the endurance cycle training session for the WC and CW training sequences (Mean, error bars represent SD).

*: WC sequence, significantly greater than previous time period (p<.05)
#: CW sequence, significantly greater than previous time period (p<.05)
5.3.4. Comparison of the Maximal Voluntary Contraction and Muscle Activation Responses for the Control Day and Sequence Days

5.3.4.1. Maximal Voluntary Contraction Peak and Mean Torque

Even though the WC sequence showed greater reductions in peak and mean torque (7.11 and 8.28%, respectively) than the CW sequence (3.55 and 4.71%, respectively) compared to the control day, which showed an increase in peak and mean torque (1.99 and 2.05%, respectively) when the respective reductions are expressed as a percentage of the pre-values, there was no main effect of trial day for peak (p=.273) or mean torque (p=.256) (Table 5.3). Whilst no main effect of time was found for peak torque (p=.082), there was for mean torque (p=.028). Post-hoc analysis indicated that there was a significant (p=.05) reduction in mean torque from the pre-1 to pre-efficiency test for the WC sequence only. No interaction of trial day by time was found for either peak (p=.328) or mean torque (p=.181).

5.3.4.2. Muscle Activation Peak and Mean Response

Comparison of the peak and mean MA responses revealed no significant difference across the three trial days however the difference did approach a level of significance (p=.062 and p=.089), respectively (Table 5.4). A significant main effect of time was found for both peak and mean MA (p=.011 and p=.019, respectively) however, post-hoc analysis did not reveal a significant difference between the three time points for any of the trial days for either peak or mean MA. There was no interaction of trial day by time for either peak (p=.870) or mean MA (p=.681), respectively.
Table 5.3. Pre the first and second training sessions and pre-efficiency test peak and mean torque for the control day and WC and CW training sequences (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Session</th>
<th>Peak Torque (Nm)</th>
<th>Mean Torque (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-1</td>
<td>Pre-2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>160.13</td>
<td>165.09</td>
</tr>
<tr>
<td></td>
<td>(37.87)</td>
<td>(36.29)</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>165.61</td>
<td>161.23</td>
</tr>
<tr>
<td></td>
<td>(35.25)</td>
<td>(40.79)</td>
</tr>
<tr>
<td>CW</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>158.76</td>
<td>158.49</td>
</tr>
<tr>
<td></td>
<td>(36.93)</td>
<td>(32.48)</td>
</tr>
</tbody>
</table>

* : Significantly less than pre-1 (p<.05)
Table 5.4. Pre the first and second training sessions and pre-efficiency test peak and mean MA for the control day and WC and CW training sequences (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Session</th>
<th>Peak Activation (%)</th>
<th>Mean Activation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-1</td>
<td>Pre-2</td>
</tr>
<tr>
<td>Control</td>
<td>86.90</td>
<td>88.44</td>
</tr>
<tr>
<td></td>
<td>(11.32)</td>
<td>(7.91)</td>
</tr>
<tr>
<td>WC</td>
<td>84.02</td>
<td>83.75</td>
</tr>
<tr>
<td></td>
<td>(8.31)</td>
<td>(5.92)</td>
</tr>
<tr>
<td>CW</td>
<td>84.56</td>
<td>83.55</td>
</tr>
<tr>
<td></td>
<td>(10.93)</td>
<td>(11.25)</td>
</tr>
</tbody>
</table>
### 5.3.5. Comparison of the Efficiency Tests Variables Post the Control Day and Sequence Days

A significant main effect of trial day was found for HR (p<.001) and NE (p=.043) for the workload period comparison and HR (p<.001) and BL (p.029) for the rest and unloaded cycling period comparison. The variable BL also approached significance (p=.073) for the workload period comparison. No significant difference was found between the trial days for $V_E$ (p=.123 and .441), $V_O_2$ (p=.064 and .407), RR (p=.139 and .084), GE (p=.912), TT (p=.295 and .223), GBM (p=.711) or RPE (p=.163) for the rest and unloaded cycling or workload period comparisons, respectively. Whilst no significant difference was found between the three trial days for a number of the efficiency test variables, the two sequence days showed elevated responses compared to the CE test. Further, the WC sequence showed greater changes than the CW sequence, as outlined below.

**Heart Rate** - post-hoc analysis indicated that the WC response was significantly (p<.05) greater than the CE response at each of the periods throughout the efficiency test (Figure 5.8). The CW response was significantly greater than the CE response but only at the 20 (p=.034) and 40% (p=.004) of $V_O_2$ max workload periods. The average increase in the HR response for the WC and CW efficiency tests compared to the control day test, was 13 and 7%, with the greatest difference for both sequences occurring at rest with 20% and 12%, reducing to 6 and 3% at the 60% $V_O_2$ max workload period, respectively. The comparison of the two sequence days revealed no significant difference except at the 20 and 60% $V_O_2$ max workload periods where the HR response for WC sequence was significantly higher than the CW sequence (p=.024 and p=.047, respectively).

**Respiratory Responses** – both the WC and CW sequence days showed elevated responses for RR, $V_E$ and $V_O_2$ above that of the CE test with an average increase of 7.3, 4.5 and 3.6% and 6.9, 4.3 and 2.9%, respectively (Figure 5.9).

**Gross and Net Efficiency** - post-hoc analysis indicated that there was no significant difference between the two sequence days for NE but there was between the CW and CE tests at the 40% of $V_O_2$ max workload period (p=.004) (Figure 5.10). Overall, both sequence days showed a small increase NE at each of the workload periods above that of the CE test.
**Blood Lactate Concentration** - the results are based on five subjects because of missing values for three of the subjects at one or more time points (Figure 5.8). Post-hoc analysis revealed a significantly higher BL concentration for the CW (p=.048) and WC (p=.043) sequences during rest and the unloaded cycling periods compared to the CE test, respectively. Even though no significant difference was found between the three trial days for the workload period comparison, the average increase in BL response for the WC and CW efficiency tests compared to the CE test, was 19.4 and 10.5%, with the greatest difference occurring at the 20% of VO\(_2\) max workload and rest with 29.4 and 26.6%, respectively.

**Tympanic Temperature** - the WC and CW sequences recorded higher TTs at each of the periods during the efficiency test than the CE test (Figure 5.11).

**Gross-Body-Mass** - a greater reduction in GBM occurred for the WC efficiency test (0.34 \(\pm\) 0.10 kg) compared to the CW (0.26 \(\pm\) 0.08 kg) and CE (0.21 \(\pm\) 0.06 kg) tests (Table 5.5).

**Rating of Perceived Exertion** - the majority of subjects reported a higher RPE during the WC efficiency test for the 40 and 60% of VO\(_2\) max workload periods compared to the CW test, which were greater than the CE test at the same workload periods (Figure 5.12).

All variables showed a significant main effect of time for the rest and unloaded cycling and workload period comparisons (p<.05) except BL (p=.084) and TT (p=.352), which showed no significant change between the rest and unloaded cycling periods. Only GBM (p=.001) and RPE (p<.001) showed a significant interaction of protocol by period for the workload period comparison, indicating that the influence of the trial day on the change in GBM and RPE was dependent upon the time of measurement. No interaction of protocol by period was found for the remaining test variables for either the rest and unloaded cycling or workload period comparisons (p>.05).
Figure 5.8. Heart rate (top) and BL concentration (bottom, n = 5) during the rest and unloaded cycling periods and the 20, 40 and 60% of VO₂ max workload periods of the efficiency test for the control day and WC and CW sequence days (Mean, error bars represent SD).

*: WC significantly greater than CE (p<.05)
#: CW significantly greater than CE (p<.05)
†: WC significantly greater than CW (p<.05)
x: Significantly greater than previous period (p<.05)
Figure 5.9. Respiratory responses RR (top), V_E (middle) and VO_2 (bottom) during the rest and unloaded cycling periods and the 20, 40 and 60% of VO_2 max workload periods of the efficiency test for the control day and WC and CW sequence days (Mean, error bars represent SD).
Figure 5.10. Gross (top) and net efficiency (bottom) during the 20, 40 and 60% of VO$_2$ max workload periods of the efficiency test for the control day and WC and CW sequence days (Mean, error bars represent SD).

# : CW sequence significantly greater than CE (p<.05)
* : Significantly greater than previous workload (p<.05)
Figure 5.11. Tympanic temperature during the rest and unloaded cycling periods and the 20, 40 and 60\% of VO\textsubscript{2} max workload periods of the efficiency test for the control day and WC and CW sequence days (Mean of seven subjects, error bars represent SD).

*: Significantly greater than 20\% of VO\textsubscript{2} max workload period (p<.05)

Table 5.5. Gross body mass pre and post the cycling efficiency tests for the control day and WC and CW sequence days (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>GBM (kg)</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>75.70</td>
<td>75.49*</td>
</tr>
<tr>
<td></td>
<td>(6.78)</td>
<td>(6.80)</td>
</tr>
<tr>
<td>WC</td>
<td>75.59</td>
<td>75.25*</td>
</tr>
<tr>
<td></td>
<td>(6.55)</td>
<td>(6.53)</td>
</tr>
<tr>
<td>CW</td>
<td>75.52</td>
<td>75.26*</td>
</tr>
<tr>
<td></td>
<td>(6.64)</td>
<td>(6.63)</td>
</tr>
</tbody>
</table>

*: Significantly less than pre (p<.05)
Figure 5.12. Rating of perceived exertion during the unloaded cycling period and the 20, 40 and 60% of VO\textsubscript{2} max workload periods of the efficiency test for the control day and WC and CW sequence days (Mean, error bars represent SD).

* : Significantly greater than previous period (p<.05)
5.3.6. Comparison of the Hormone Responses for the Control Day and Sequence Days

The results for both testosterone and cortisol are based on the values obtained from five of the eight subjects due to missing samples for two subjects (refer Section 5.2.4.2 Blood Specimen Collection) and extreme values recorded for the remaining subject (greater than $±1.82$ SD from the mean), as determined by the Grubbs method (Zhang, 1986).

5.3.6.1. Testosterone

Even though the serum testosterone response was similar for all trial days with a reduced concentration for the post-training compared to the pre-training time point (Figure 5.13), a significant main effect of trial day ($p=.018$) and time ($p=.016$) was found. There was also a significant interaction of trial day by time ($p=.019$) indicating that the influence of the trial day on the testosterone response depended on the time of sampling.

Post-hoc analysis revealed that the WC sequence pre- and post-training values ($9.70 ± 0.26$ and $8.12 ± 0.82$ ng.mL$^{-1}$) were significantly higher than the control ($8.80 ± 0.62$ ng.mL$^{-1}$, $p=.022$) day at the pre-time point and significantly higher than the CW sequence ($6.94 ± 1.06$ ng.mL$^{-1}$, $p=.024$) response at the post-training time point, respectively. There was no significant difference between the control day and CW sequence response for either the pre- or post-training time points or between the sequence days for the pre-training time point ($p>.05$). The control day and WC and CW sequence days all showed a significant reductions in serum testosterone from pre- to post-training ($p=.007$, $p=.012$, $p=.010$, respectively).

5.3.6.2. Cortisol

Cortisol showed a similar response pattern to testosterone with reduced serum concentrations for the post-training compared to the pre-training time point for all trial days (Figure 5.13). However, unlike testosterone, no significant difference was found between the control day and the WC and CW sequence days ($p=.810$). A significant main effect of time ($p=.003$) was found but there was no interaction of trial day by time ($p=.525$).

There was a significant ($p=.002$) reduction in serum cortisol concentration from $413.46 ± 78.98$ to $269.13 ± 54.49$ ng.mL$^{-1}$ and from $412.94 ± 80.21$ to $303.77 ± 78.44$ ng.mL$^{-1}$ over the...
duration of the day for the WC and CW sequences, respectively. However, there was no significant (p=.140) difference between the control day pre- (385.31 ± 32.84 ng.ml⁻¹) and post-training time points (306.49 ± 76.07 ng.mL⁻¹).

5.3.6.3. Testosterone/Cortisol Ratio

Even though the WC sequence showed a greater ratio change compared to the control day and CW sequence day (Figure 5.14), no significant difference was found between the three trial days (p=.252). There was also no main effect of time (p=.160) or interaction of trial day by time (p=.512) found.
Figure 5.13. Testosterone (top) and cortisol (bottom) responses over the duration of the day for the control day and WC and CW sequence days (Mean of five subjects, error bars represent SD).

* : Significantly different from pre (p<.05)
# : Significantly different from control day pre (p<.05)
† : Significantly less than WC post (p<.05)
Figure 5.14. Testosterone/cortisol ratio over the duration of the day for the control and WC and CW sequence days (Mean of five subjects, error bars represent SD).

5.3.7. Summary of Results

In summary,

- The W session of the CW sequence resulted in a significantly higher pre and higher post-training BL concentration compared to the WC sequence. The C session of the WC sequence showed a higher physiological cost of cycling than the CW sequence with higher respiratory responses (RR, VE, VO₂), HR, BL, TT and NE whilst GE was notably lower. No significant difference was found between the two sequence days for CK.

- The W session of the CW sequence produced a similar reduction in peak and mean knee-extension torque to that of the WC, respectively. In contrast, the C session of the WC sequence showed a greater reduction in peak and mean torque than that found for the CW sequence, which showed no change in peak and < 1.5% reduction in mean torque. The result was a significantly greater reduction of 8.28%
from pre-training to three-hours post-training for the WC sequence compared to the CW sequence. No significant difference was found across the three control day time points for MVC or between the three trial days for MA.

- Whilst no significant difference was found between the three trial days for a number of the efficiency test variables, both sequence days showed elevated responses compared to the CE test. Furthermore, the WC sequence showed greater changes than the CW across the majority of the variables measured during the efficiency test including HR, BL, RR, $V_E$, $VO_2$, GBM and RPE.

- A significantly lower post-training testosterone concentration was found for the CW compared to the WC sequence whilst a significant reduction in cortisol was found for the WC and CW sequence days compared to the control day. This resulted in a lower post-training T/C ratio for the CW sequence compared to the WC sequence.
5.4. Discussion

5.4.1. Introduction

Single (e.g. cyclists, runners and swimmers) and multi-discipline athletes (e.g. duathletes and triathletes) are known to train up to three times per day with short intervals of 2-3 hours in between various sessions of strength and endurance training. Whilst the completion of such training regimes is commonplace in sporting arenas, the question is whether there is an optimal order that the training sessions should be completed in, ie. whether strength training should be performed before endurance, or visa versa? Furthermore, whether short recovery periods of three hours or less between concurrent training sessions are sufficient to provide complete recovery before commencing another training session? In view of the limited research that has addressed these issues of training sequence and frequency, the overall aim of Study 2 was to examine the acute residual physiological effects of two sequences of strength and endurance training completed on the same day on muscle force generating capacity and cycling efficiency three hours post-training. The major finding of this study was that there is a residual effect of prior training sessions on subsequent training sessions with those training sessions completed second in the training sequence being completed at a higher physiological cost compared to when the same training sessions were completed first. In addition, that the degree of this effect is dependent on the sequence of training, with the WC sequence showing a greater reduction in MVC torque prior to and increased physiological cost during the efficiency tests following the training sessions compared to the CW sequence.

5.4.2. Comparison of the Maximal Voluntary Contraction and Muscle Activation Trials for the Control Day and Sequence Days

Due to the sequence days for Study 2 being conducted over an eight-hour period, a control day was included in the study design for the MVC and MA trials to ensure that any changes that occurred over this time period were not due to diurnal fluctuations in voluntary strength or MA, similar to that found for other body systems e.g. endocrine (Hackney, Premo, & McMurray, 1995; Ronsen et al., 2001). No significant difference was found between the three test periods (Pre-1, Pre-2 and Pre-Efficiency) for both peak and mean torque and voluntary MA during the control day. This suggests that the MVC and MA trials were stable over the course of the day and as such any changes in the MVC or MA trials during the sequence days may be attributed to other factors other than diurnal variations in strength or muscle
activation. No significant difference was found between the control day and two sequence days for either peak or mean torque, however there was a notable pattern of change following the training sessions for the sequence days.

The W session produced a greater reduction in peak and mean torque (3.43 and 3.68%, respectively) when expressed as a percentage of pre-training values than the C session, which showed no change in peak torque and a negligible 1.24% reduction in mean torque, when the training sessions were completed first in each training sequence in the present study. The corresponding voluntary MA indicated that the reduction for the W session was not due to a reduction in MA, as no significant change was found from Pre-1 to Pre-2. However, as was the case with the results of Study 1, the voluntary MA disguised the fact that there was a reduction of ~7% in both the superimposed response and the control response for the W session of the WC sequence compared to the C session of the CW sequence, which showed less than 1% change. Based on these changes in both the superimposed and control twitches, the reduction in torque following the W training session was due to some form of peripheral fatigue (Bigland-Ritchie et al., 1986). These present findings indicated that the level of fatigue following the 60-minute C session was less than that of the 60-minute W session and/or that the rate of recovery was faster for the C session compared to the W session. This difference may have been due to the intensity of the respective training sessions, as the W session involved high-intensity (85% of 1 RM) loads whilst the C session was performed at a moderate intensity (60% of VO₂ max), a factor known to influence the level of fatigue post-exercise (Häkkinen, 1992). The difference could have also been due to the type of exercise, as the W session involved eccentric and concentric contractions whilst the C session involved only concentric contractions, which has also been shown to effect the level of fatigue post-exercise (Folland et al., 2001; Newham, Jones et al., 1983).

The reduction in torque for the W session of the WC sequence is consistent with previous reports of a reduction in force generating capacity following high-intensity strength training in trained subjects (Häkkinen, 1990; Häkkinen, 1992, 1993). However, the responses of the C session differ to previous investigations of force generating capacity post-endurance cycling exercise (Bentley et al., 1998; Sahlin & Seger, 1995). Bentley et al (1998) reported a significant reduction in force generating capacity six hours post-exercise after 30 minutes of cycling at the lactate (D_max) threshold including four 60-second efforts at 120% of VO₂ max. Sahlin and Seger (1995) reported that force-generating capacity was only 80% of pre-exercise
values 30 minutes following ~ 90 minutes cycling at 75% of VO₂ max. Whilst it is difficult to compare the present findings to these studies due to different testing time points, protocols and types of subjects examined, the reduced exercise intensity and/or duration of the C session in the present study may explain why the reduction in torque following the C session was not significant or as dramatic as that reported by Bentley et al (1998) and Sahlin and Seger (1995).

A similar finding to that described above for the W session of the WC sequence was also found for the CW sequence when the W session was preceded by the C session, with a reduction in peak and mean torque of 3.55 and 4.71% when the reduction in torque is expressed as a percentage of pre-training values. The superimposed and control twitch responses also showed a similar reduction to that outlined for the WC sequence (6.89 and 6.66%, respectively), which again indicates some form of peripheral fatigue (Bigland-Ritchie et al., 1986). These similarities in torque reductions and twitch responses between when the W session proceeded and followed the C session indicates that the C session did not have a substantial negative effect on the recovery of force generating capacity post-exercise. If so, then a greater reduction in torque should have occurred. In contrast, the C session for the WC sequence showed a greater reduction in peak (3.68%) and mean torque (4.6%) than that found for the C session of the CW sequence, which showed no change in peak torque and a minimal change in mean torque. The reduction in mean torque for the C session resulted in a significant reduction of 8.28% from the Pre-1 to the Pre-Efficiency time point for the WC sequence compared to the CW sequence, which showed a non-significant 4% reduction. This indicated that the subjects during the C session of the WC sequence experienced fatigue that was not found during the CW sequence. It also suggests that the fatigue was mainly caused by the W session.

This study is the first to compare the recovery of force generating capacity throughout a concurrent training session. As such comparison to other studies is difficult. However, the change in fatigue characteristics for the C session of the WC sequence are in agreement with reports of altered fatigue following a second high-intensity strength training session of equal training volume completed on the same day (Häkkinen, 1992). Häkkinen (1992) reported a significantly greater reduction in maximal strength along with a significant shift in the average force-time curve from the first to the second sessions in nine male athletes even though they had recovered to pre-session one strength levels prior to the commencement of
the second training session. The completion of two strength training sessions is different to completing a strength and endurance training sessions concurrently, however, the findings by Häkkinen (1992) do suggest that the fatigue mechanisms contributing to the first training session may be compounded by the second session or that the mechanisms contributing to fatigue following the second session are different to those of the first session.

Reductions in force generating capacity are often attributed to muscle damage (Enoka, 2002; Folland et al., 2001). However, the CK response post-training for the two training sequences in the present study indicated that the sequence of training did not affect the overall level of muscle damage, as no significant difference was found between the two training sequences 24 hours post the W session. Whilst the CK response post-training only provides a marker for possible damage to muscle (Hortobágyi & Denahan, 1989; Newham et al., 1987; Nosaka & Clarkson, 1992), the results suggest that the difference in torque after the training sequences may not have been due to damage to muscle contractile structures. Furthermore, the CW sequence did not cause any more damage than the WC sequence, even though a greater level of stress was incurred during the W session of the CW sequence, as indicated by the notable increase in BL concentration post-training. Whilst these findings imply that mechanisms other than damage to muscle contractile structures may have contributed to the reduction in torque post the C session of the WC sequence, they do not preclude muscle damage as a mechanism, just that the level in this study, as indicated by the CK response, may have been insufficient to be an influencing factor.

5.4.3. Comparison of the Weight and Endurance Cycle Training Sessions

It is well documented that the level of force production is maintained in periods of fatigue by the additional recruitment of large, more powerful glycolytic fast-twitch motor units (Sale, 1987; Wilson, 1994a). Further, it is well documented that the increased recruitment of motor units means an increased impedance of muscle blood flow due to a higher level of muscle contraction (Fleck, 1988; Fleck et al., 1989; St Clair Gibson et al., 2001), which can contribute to a higher level of BL accumulation due to an increase in local ischemia as well as a reduction in lactate removal (Lambert & Flynn, 2002; Sawka, 1986; Takaishi, Yasuda, & Moritani, 1994). The current findings for the C session of the WC sequence supports these suggestions, as a significant increase in BL concentration (~30%) was found post-training compared to the C session of the CW sequence. The WC sequence also showed a higher mean
RER throughout the C session compared to the CW sequence, indicating a shift towards anaerobic metabolism (McArdle et al., 1996). The increased BL concentration indicates an increased level of effort was required to complete the C training session when completed after a prior bout of strength training and is consistent with the reduction in peak and mean torque found for MVC trials. The W session of the CW sequence also showed a significant increase in BL concentration post-training (~20%). However unlike the WC sequence, the C session of the CW sequence showed minimal change in peak or mean torque, hence no suggestion of fatigue post-exercise. This suggests that another mechanism(s) contributed to the result other than fatigue, possibly altered motor unit recruitment patterns due to the difference in movement pattern for the two training sessions (Bangsbo, 1996; Ebbeling & Clarkson, 1989).

An increased contribution of anaerobic metabolism during a bout of exercise performed after a bout of fatiguing exercise, using the same muscle groups, has been reported previously (Gleeson, Blannin, Walsh, Field, & Pritchard, 1998). Gleeson et al (1998) reported a significantly higher BL concentration during sub-maximal cycling exercise two days after a prior bout of bench stepping exercise. The current findings and those of the above study suggest that the performance of an activity that uses the same muscle groups as those used earlier in the day or previous days, irrespective of whether the activity is strength or endurance orientated, may increase the level of contribution of anaerobic metabolism due to the presence of an element of fatigue or possible disruption to motor unit recruitment patterns. It must be pointed out that the post C session BL concentrations for both training sequences indicated a low-moderate intensity and as such it is possible that a greater effect on the W session of the CW sequence may have been found had the intensity of the C session been higher and similar to that of Bentley et al (1998) and Sahlin and Seger (1995).

In view of the significant increase in BL concentration for the C session of the WC sequence, it was anticipated that the other physiological variables would also be elevated above the C session responses for the CW sequence. Heart rate during the C session for the WC sequence was significantly greater than the CW sequence for the rest and warm-up periods and whilst the difference progressively reduced as the duration of the exercise proceeded, was still elevated above the CW sequence at the end of the session. Similarly, the respiratory responses RR, VE and VO₂ for the WC sequence were also elevated above that of the CW sequence both at rest and during exercise. These elevated resting values indicate the subjects were still in the process of returning body processes to their pre-exercise state when the second training
session commenced. The significance of this elevated state is that if processes are not restored prior to the commencement of a second training session than a greater physiological cost might occur during the second session as the body attempts to cope with the residual demands of the prior training stimulus as well as the physiological demands of the new training stimulus.

The efficiency results for the C session of the WC sequence for the present study confirmed the presence of an increased physiological cost, as GE was notably less than the C session of the CW sequence. In addition, the fact that there was also a significant decline in GE for the WC sequence from the 11-20 to 51-60 minute periods also highlights that the physiological cost of the exercise was increasing as the session progressed compared to the CW sequence, which showed no real change over the same time period. Whilst the present study is the first to examine the acute physiological responses following sequences of strength and endurance training on the same day, the results support the findings of Ronsen et al (2001) who reported an increase in VO$_2$ during a second training bout of endurance exercise conducted three hours after a 65-minute training bout of endurance exercise at 75% of VO$_2$ max. Similarly, the present results support the findings of Bahr et al (1991) who reported an increase in oxygen consumption both at rest and during sub-maximal cycling of 14 and 19%, respectively and a reduction in NE from ~25% to ~21%, compared to a control trial following insufficient recovery between exercise sessions.

### 5.4.4. Comparison of the Efficiency Tests Post the Control Day and Sequence Days

In light of the two training sequences (WC and CW) showing reductions in peak and mean torque at the pre-efficiency time point compared to the control day, that showed no change over the duration of the trial period, one might anticipate that a greater physiological response would be found for the efficiency tests following the training sequences compared to the control day. Further, because of the greater reduction in peak and mean torque for the WC sequence compared to the CW sequence at the pre-efficiency time point, that a difference would be found between the sequences for the efficiency test variables. The trends in the results support the above lines of reasoning. However, the extent of the physiological responses and difference between the control and sequence days was small and not statistically significant for a number of the efficiency test variables including RR, $V_E$, VO$_2$, TT, GBM and RPE.
Both training sequences showed elevated physiological responses across both the rest and unloaded periods as well as the workload periods of the efficiency test compared to the CE test, with the most notable differences occurring for the physiological variables HR, and BL and to a lesser extent the respiratory responses, TT, GBM and NE. The elevated resting responses for the sequence days above that of the CE test indicated that there was a residual effect from the prior training sessions on resting metabolism. Both sequences showed that this residual effect continued throughout the exercise periods similar to the increased physiological cost found for the C session of the WC sequence. However, what was different about the efficiency test responses compared to the C session following the W session, was that the WC sequence consistently produced non-significant higher physiological responses than the CW sequence even though when the W session was completed first in the training sequence it produced a marked effect on the physiological responses of the following C session. This suggests that the proximity of the higher intensity training session (W session) to the efficiency test was not the determining factor in the duration of the residual effect of the training sessions but more the combination of the prior W session and the subsequent C session. This finding is consistent with the greater reductions in torque found for the WC sequence compared to the CW sequence as well as the higher BL concentrations found throughout the WC efficiency test compared to the CW sequence, indicating a greater contribution of anaerobic metabolism to maintain power output.

Generally, increases in BL concentration are reflected by a change in GE and NE due to an increase in energy expenditure (Brooks et al., 2000). The reason why a difference in efficiency was not found between the two training sequences as well as the control day in the present study, similar to that found for the C session following the W session, may have been due to the level of BL produced during the 20, 40 and 60% of VO₂ max workloads for the efficiency test (e.g. WC sequence, 1.13 ± 0.20, 1.55 ± 0.22 and 2.33 ± 0.21 mmol.L⁻¹, respectively) and the small differences between the three trial days. These levels being of insufficient concentration to significantly alter the respiratory responses. However, the fact that HR for the WC sequence was significantly elevated above that of the CW sequence yet the difference between the training sequences was less notable for the respiratory responses of GE and NE, suggests that the recovery dynamics for HR and respiratory responses may be different. Short and Sedlock (1997) previously observed that HR and VO₂ remained elevated throughout the recovery period but recovered at different rates. At present there are no
explanations for this phenomenon but possible reasons include altered circulatory dynamics, 
prolonged sensitivity of cardiac tissues to elevated temperature, hormones or metabolites, or 
mild psychological arousal induced by the activity (Short & Sedlock, 1997).

A small number of studies have examined the effect of the sequence of concurrent training 
sessions on strength and endurance adaptations (Bell et al., 1988; Bell et al., 1991b; Collins & 
Snow, 1993; Gravelle & Blessing, 2000). Three of the four studies reported impedance of 
either strength or VO₂ max compared to a control group or the other sequence group whilst 
only one reported no difference between training sequences (Collins & Snow, 1993). The 
findings of the three studies that found differences indicate that strength and endurance 
adaptation are dependent on the sequence in which concurrent training sessions are 
completed. Whilst the current study did not examine adaptations to training, the findings 
showed that like the training studies (Bell et al., 1988; Bell et al., 1991b; Gravelle & Blessing, 
2000), the sequence of training does affect the responses of the individual training sessions, 
which could influence the level of adaptation over time. Furthermore, the current findings 
indicate that the order in which concurrent training sessions are completed is important in 
determining the overall level of fatigue post-exercise and/or the rate of recovery.

5.4.5. Comparison of the Hormone Responses Post the Control Day and Sequence 
Days

The hormones testosterone and cortisol were measured prior to commencing the first training 
session and the efficiency test in order to determine if the sequence of training altered the 
hormonal state of the subjects at a common point in time during recovery from the two 
training sessions. The results of this study indicate that the sequence of training does alter the 
hormonal response post-training and has the potential to change the ratio of 
testosterone:cortisol (T/C).

In the present study, the reduction in testosterone and cortisol from pre-training in the 
morning to the pre-efficiency time point in the afternoon for both the control and sequence 
days was consistent with previous reports that serum testosterone and cortisol concentrations 
gradually decline over the duration of the day irrespective of whether exercise has been 
performed earlier in the day or not (Hackney et al., 1995; Ronsen et al., 2001). Whilst no 
significant difference was found between the control and WC sequence day post-training for
testosterone, a significantly lower concentration was found for the CW sequence compared to the WC sequence. This indicated that the sequence of completing a high-intensity W session three hours after an endurance C session evoked a poorer testosterone response than if completed prior to the C session. In comparison, the serum cortisol concentration post-training for the two sequence days was not significantly different from that found for the control day and would suggest that the sequence of training does not alter the cortisol response post-training, as reflected by this time point.

Whilst the hormonal response in the hours following these time points in the present study are not known, the results do pose the question of which order is going to provide the best response for strength and/or endurance adaptation? The answer to this question is provided in the form of the T/C ratio, as changes in the hormonal environment may affect processes at the cellular level, such as muscle fibre adaptations (e.g. hypertrophy) via changes in protein synthesis (Kraemer et al., 1995) in addition to altering substrate utilisation (Chattoraj & Watts, 1986; Galbo, 1992; Kraemer, 2000b). The results of the T/C ratio did not reveal any significant difference between the control and sequence days. However, the CW sequence (0.025 ± 0.010) did show a lower ratio than the WC sequence (0.031 ± 0.008), which is representative of a more catabolic state for the CW sequence compared to the WC sequence. Whether this level of change in the hormonal environment is capable of altering strength and endurance adaptations in the long-term is unknown. However, it does indicate that the sequence of training has the potential to alter the balance between anabolic and catabolic processes, measured via changes in testosterone and cortisol responses (Adlercreutz et al., 1986; Banfi, 1998). This finding and that of previous investigations of multiple training sessions in one day (Häkkinen et al., 1988; Ronsen et al., 2001), which reported altered hormonal responses following a second training session on the same day, adds support to the concept that changes in the hormonal environment during concurrent training regimes may contribute to the alterations in strength and endurance adaptations (Bell et al., 2000; Leveritt et al., 1999).

It should be pointed out though that the current findings might have been influenced by the proximity of the second training sessions to the sampling time point. Multiple set strength training protocols have been shown to evoke notable increases in serum testosterone concentration immediately following training (Gotshalk et al., 1997), with the concentration during the recovery period gradually declining (Fellmann et al., 1989; Kindermann et al.,
1982; MacConnie et al., 1986; Ronsen et al., 2001; Tabata et al., 1990; Viru et al., 1992). Similarly, endurance training has been shown to affect the testosterone (Semple et al., 1985; Urhaausen & Kindermann, 1987) and cortisol responses to varying degrees post-exercise (Fellmann et al., 1989; Kindermann et al., 1982; MacConnie et al., 1986; Ronsen et al., 2001; Tabata et al., 1990; Viru et al., 1992).

The limitation of using only two data collection points over a short period of time (8 hours), as used in this study, is that the magnitude and time course of the hormone responses both immediately following the different training sessions as well as over the recovery period following training (i.e. the first 24 hours) is not known. These changes (magnitude and time course) may hold the key to better understanding how possible alterations in the endocrine response influence the level of adaptation post-training hence the need for a greater frequency and duration of sampling throughout the sequence days, an issue that is addressed in more detail in Study 3.

5.4.6. Summary and Conclusion

In summary,

• The sequence of completing concurrent training sessions affected the recovery of force generating capacity between and following the training sessions, with a greater level of fatigue incurred for the WC sequence compared to the CW sequence. The completion of a W session prior to the C session attenuated the fatigue normally found following a C session conducted without a preceding W session.

• The level of muscle damage incurred by subjects, as assessed by changes in CK activity, did not appear to be affected by the sequence that the W and C training sessions were completed. Furthermore, that the reduction in force generating capacity during and following the two training sequences seemed to be due to different fatigue mechanisms.

• Irrespective of the sequence of training, the completion of a second training session three hours after a prior training session increased the level of
physiological stress of subjects during that session, requiring a greater contribution from anaerobic metabolism to maintain power output. The increased physiological stress during the C session of the WC sequence compared to the CW sequence also caused an increase in energy expenditure, resulting in a reduction in GE.

- A longer recovery period than three hours was required between and following the training sessions in order to allow complete recovery from the proceeding training session. Further, that the WC sequence required a greater recovery period than the CW sequence following the second training session, as evident by the increased physiological stress during the efficiency test.

- The hormonal responses three hours post the concurrent training sessions were affected by the order in which the training sessions were completed, with a more catabolic state found for the CW sequence compared to the WC sequence, as reflected by the lower T/C ratio.

It was concluded that the recovery of force generating capacity and physiological parameters during and post-exercise are dependent on the sequence of training, with the WC sequence having a greater affect than the CW sequence. This finding supported the hypothesis outlined in Chapter 1, as to the finding that there was an increased physiological stress for the W and C sessions when completed second in the training sequence compared to when the same training sessions were completed first. Therefore, consideration must be given to the order in which training sessions are performed and the scheduling of exercise bouts in designing concurrent training regimes where multiple training sessions are completed in the same day, so as to limit the possible negative residual effects of proceeding exercise and optimise training adaptation.
CHAPTER 6

Study 3

The effect of the sequence of strength and endurance training on hormonal and gene expression responses and muscle glycogen content post-training

6.1. Introduction

Both acute and chronic mechanisms have been proposed to explain the strength and/or endurance impedances found in concurrent training regimes (Chromiak & Mulvaney, 1990; Leveritt et al., 1999). The acute mechanism referring to residual fatigue from such sources as neuromuscular fatigue, muscle damage and muscle glycogen depletion whilst the chronic mechanism refers to alterations in the concentrations of various anabolic/catabolic hormones, differences in the organisation of neuromuscular recruitment patterns and changes in muscle fibre transformation and hypertrophy processes (Chromiak & Mulvaney, 1990; Leveritt et al., 1999). There has been speculation as to how some of these mechanisms may be altered, however one source may be changes in hormonal or gene expression responses due to altered mechanical or metabolic stresses from the concurrent training sessions. Even though it is unlikely that any one of the proposed mechanisms is solely responsible for interfering in strength and/or endurance development during concurrent training regimes, the role that each proposed mechanism plays in the complex interaction of training, response and adaptation, is unknown.

The aim of the this study was to investigate some of the proposed mechanisms mentioned above by determining whether the sequence of completing strength and endurance training sessions on the same day altered:

- the hormonal responses of testosterone and cortisol compared to single mode training sessions post-training;
• the level of skeletal muscle glycogen depletion and recovery, during and following the training sessions, respectively; and

• the level of expression of selected genes associated with skeletal muscle growth and metabolism, post-training.

6.2. Methods

6.2.1. Study Design

Following a familiarisation session and two assessment trials (leg strength and VO₂ max) as described in the General Methodology and Materials Chapter (Sections 3.6. and 3.7), the subjects completed four trial days (two control and two sequence days) over a five-week period (Table 6.2).

Table 6.2. Project design for the four trial days of the study.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training Protocol</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Day 1</td>
<td>Weights (W)</td>
<td>1</td>
</tr>
<tr>
<td>Control Day 2</td>
<td>Cycle (C)</td>
<td>2</td>
</tr>
<tr>
<td>Sequence Day 1</td>
<td>Weights then Cycle (WC)</td>
<td>3</td>
</tr>
<tr>
<td>Sequence Day 2</td>
<td>Cycle then Weights (CW)</td>
<td>5</td>
</tr>
</tbody>
</table>

There was a minimum of three days between the familiarisation session and VO₂ max test and seven days between the VO₂ max test and the first control day, in order to eliminate any possible carry-over effects from one test to another. Further, there were 14 days between the two sequence days in order to eliminate any possible carry-over effects from one test to another and also to allow the biopsy wounds sufficient time to completely heal. All trial days were carried out on the same day of the week.
The subjects were randomly allocated to one of two testing orders, with four subjects completing each order.

Order 1.  W  C  WC  CW

Order 2.  C  W  CW  WC

6.2.1.1. Description of the trial days

The subjects arrived at the laboratory at 07:00 hours at which time pre-training blood and muscle specimens were collected (Note: muscle specimens were not collected during the control days). The subjects then rested for an hour before completing either a one-hour W or three-hour C training protocol at 08:00 hours, as outlined below. Following the training session, the subjects rested: 1) for the remainder of the day to 18:00 hours (control days) or 2) for three hours (sequence days), during which time blood and blood/muscle specimens were collected, respectively. After the three-hour rest period, the subjects then completed the second training protocol (C or W, respectively), both of which finished at 15:00 hours. Following the second training session, the subjects rested for the remainder of the day to 18:00 hours. Twenty-four hours after the pre-training blood specimen and the pre W session muscle specimen time points, the subjects returned to the laboratory to provide another blood and muscle specimen, respectively (Figure 6.1A and 6.1B).

Throughout the duration of the control and sequence days, the subjects were required to remain within the laboratory facility and rest (remained seated) when not training, by undertaking such activities as reading or watching television. Dietary intake was standardised throughout the trial days as described in the ‘Subject Preparation’ section in Chapter 3.
Training and Muscle Biopsy Schedule

Control Day 1

Sequence Day 1

Blood Sampling Schedule

Figure 6.1A. Study Design: Control Day 1 is paired with the corresponding Sequence Day 1. The dark and light shaded areas refer to the W and C sessions, respectively. The large and small arrows reflect the muscle biopsy and blood sampling time points, respectively. The long small arrows for the Blood Sampling Schedule refer to those sampling time points applicable only to the sequence day.
Training and Muscle Biopsy Schedule

Control Day 2

Sequence Day 2

Blood Sampling Schedule

Figure 6.1B. Study Design: Control Day 2 is paired with the corresponding Sequence Day 2. The dark and light shaded areas refer to the W and C sessions, respectively. The large and small arrows reflect the muscle biopsy and blood sampling time points, respectively. The long small arrows for the Blood Sampling Schedule refer to those time points applicable only to the sequence day.
6.2.2. Subjects

Eight (8) male athletes who were actively participating in endurance training (cycling, running or rowing) and weight training on a regular basis, volunteered for the study. Their mean (±SD) age, height, body mass and VO₂ max (absolute and relative) are outlined in Table 6.1.

Table 6.1. Descriptive characteristics of the eight subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.1 ± 2.4</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.80 ± 3.49</td>
</tr>
<tr>
<td>Body Mass (kilograms)</td>
<td>77.19 ± 10.70</td>
</tr>
<tr>
<td>VO₂ max Absolute (L.min⁻¹)</td>
<td>4.49 ± 0.48</td>
</tr>
<tr>
<td>VO₂ max Relative (mL.kg⁻¹.min⁻¹)</td>
<td>58.93 ± 8.92</td>
</tr>
</tbody>
</table>

The volunteers were selected if they met the following criteria.

- Aged between 18 and 40
- Performing endurance training (i.e. cycling, running or rowing) a minimum of 2-3 times per week including at least two cycling sessions per week with an approximate weekly duration total of 3-4 hours
- A minimum of 12 months weight training experience
- Currently performing weight training exercises a minimum of twice per week
- No contraindications to the test procedures.

6.2.3. Training Protocols

For the control and sequence days, the subjects completed one or both of the following training protocols, respectively.
**Weight (W) Training**

**Warm-up:** Five minutes of stationary cycling at a relative workload corresponding to 1 watt per kg of GBM followed by two sub-maximal trials at 45% and 65% of leg-press and 50% and 70% of leg-curl and -extension 1 RM and 6 RM for ten and five repetitions, respectively.

**Training Sets:**
- Leg-press 6 x 6 RM
- Leg-curl 4 x 6 RM
- Leg-extension 4 x 6 RM

The 6 RM loads were calculated as 85% of each subject’s leg-press 1RM (Feigenbaum & Pollock, 1997; Hoeger et al., 1990) and 100% of each subject’s leg-curl and -extension 6 RM.

**Rest Intervals:** One-minute between warm-up trials and three-minutes between training sets and exercises

**Duration:** 60 minutes

**Cycle Endurance (C) Training**

**Warm-up:** After completing five minutes of rest sitting on the cycle ergometer the subjects completed a warm-up of five minutes of cycling at a workload corresponding to 40% of VO2 max

**Grade:** Level

**Cadence:** 90 rpm

**Intensity:** Workload corresponding to 60% of VO2 max

**Duration:** 170 minutes
6.2.4. Sampling Protocol and Measurements

In order to determine if the sequence of training affected hormonal and skeletal muscle gene expression responses as well as muscle glycogen content, a number of specimens (blood and skeletal muscle) were collected prior to and following the C and W training sessions, as outlined below. Physiological variables were also measured during the training sessions including respiratory responses, HR and RPE. The specimens and variables were collected and analysed as described in the corresponding section in the General Methodology and Materials (Chapter 3).

6.2.4.1. Blood Specimen Collection

Venous blood specimens were collected at the following time periods on both the control and sequence days:

- one-hour before the commencement of the first training session (07:00 hours),
- immediately following each training session,
- every 15 minutes for the first hour immediately following each training session and then every hour thereafter for the next three hours, and
- 24 hours after the initial trial day’s resting morning specimen,

for the determination of resting and pre- and post-training,

- blood lactate concentrations, and
- plasma cortisol and testosterone concentrations.

The subjects were laid supine on a bed in a quiet room to rest 20 minutes prior to the initial control and sequence days’ 07:00 hours specimen collection time. With respect to the post-exercise specimens, all the specimens were collected within 60 seconds of completing each training session and before dismounting the cycle ergometer or the leg-extension machine in the C and W sessions, respectively. The same fast and sampling procedure was employed for the specimens taken the morning following the control and sequence days.

A total of 13 and 16 venous blood specimens were collected from the subjects on the control and sequence days, respectively (Figures 6.1A and 6.1B). Three less specimens were
collected for the control days due to the 15-minute interval specimens not being collected during the hour immediately following the time period corresponding to the second training session on the sequence days.

6.2.4.2. Muscle Specimen Collection

Skeletal muscle specimens were collected at the following time intervals on the sequence days only:

- one-hour before the commencement of the first training session,
- immediately following each C session and one-hour post each W session,
- immediately prior to the commencement of the second training session,
- three hours after the completion of the second training session, and
- 24 hours after the one-hour post W session sample,

for the determination of resting and pre-and post-training

- muscle glycogen content, and
- muscle mRNA content.

The initial sequence days’ 07:00 hours specimens were collected immediately following the venous blood specimen collection, as described above. With respect to the post C session specimens, all the specimens were collected immediately upon dismounting the cycle ergometer and the application of a local anaesthetic. The post W session specimens were delayed one-hour because prior research data from the same laboratory had shown that some of the genes to be examined in this study responded more in the hours following strength training than immediately post-training (Psilander, 2002). A total of 12 muscle biopsies were taken per subject, six on each sequence day (Figures 6.1A and 6.1B).

6.2.4.3. Respiratory Gases

Expired respiratory gases were collected during the five-minutes rest and warm-up periods and throughout the C session in 10-minute periods every 20 minutes, starting 20 minutes into the 170-minute training session (i.e. at 20-30, 50-60, 80-90, 110-120, 140-150 minutes). Because collection occurred for 10 minutes out of every 30 minutes, the last sampling period
was completed 20 minutes prior to the completion of the session due to the length of the C session being 170 minutes. Further, only the last five minutes of each period was used for reporting purposes. The measuring of respiratory gases in intervals allowed for fluid intake at regular intervals throughout the training session, as described in the General Methodology and Materials Chapter (Section 3.7.3). The same respiratory variables and method of calculation of gross and net efficiency, as reported for the C training sessions in Study 2 were used in Study 3.

6.2.4.4. Heart Rate

Heart rate was recorded continuously throughout the W and C sessions for both the control and sequence days. Heart rate is reported for the same five-minute intervals as used for the respiratory responses during the C sessions.

6.2.4.5. Rating of Perceived Exertion

The RPE scale was used to ascertain each subject’s perception of effort during the C sessions and recorded in the last 15 seconds of the 10-minute expired respiratory gas collection period.

6.2.5. Statistical Analysis

Descriptive statistics (mean and SD) were calculated for all measured variables except the gene expression analysis were the mean and standard error was used.

6.2.5.1. Comparison of the Weight and Endurance Cycle Training Variables

A three-factor repeated measures ANOVA with two-within subject factors, training sequence (WC and CW) and time (pre- and post-training) and one-between subject factor, test order was used in order to determine those training variables that responded differently to the training sequences. This analysis was completed for the weight and endurance cycle training dependent variable BL concentration.

A three-factor repeated measures ANOVA with two-within subject factors, training sequence (WC and CW) and exercise (leg-press, -curl and -extension) and one-between subject factor,
A three-factor (trial day by period by test order) repeated measures ANOVA was used to determine if the dependent variable peak and mean HR responded differently to the training sequences. The analysis of the endurance cycle training variables was completed by splitting the analysis into two due to the number of data points, similar to the statistical analysis method used for the efficiency test variables in Study 1 and 2. For those variables with more than three sample points, two comparisons were completed: (one) rest and warm-up, (two) 25-30, 55-60, 85-90, 115-120 and 145-150 minute time periods. This analysis was completed for the dependent variables HR and RPE.

### 6.2.5.2. Comparison of Muscle Glycogen Content for the Sequence Days

A repeated measures ANOVA with two-within subject factors, training sequence (WC and CW) and time (pre and post W, pre and post C, three hours following the second training session and the 25 hours post the W training session) and one-between subject factor, test order was used to determine if there was a difference between the training sequences over the course of the trial days.

### 6.2.5.3. Comparison of the Hormone Responses for the Control and Sequence Days

In view of the large number of sample time points from the initial pre-trial day 07:00 hour (referred to as “pre 07:00” in the text) to the following mornings 07:00 hour (referred to as “post 07:00” in the text) for the control and sequence days as well as the limited number of subjects, it was not possible to statistically analysis all the samples within the one analysis and as such three comparisons were made using a three-factor (training sequence by time by test order) repeated measures ANOVA.

**Comparison A** = those time points from the pre 07:00 sample up to and including the time corresponding to the commencement of the second training session. This comparison was completed to determine if the various trial days altered the hormonal response at selected common time points prior to the commencement of the second training session.
Comparison B = those time points immediately after the time corresponding to the completion of the second training session (15:00 hours) up to and including the 18:00 hour time point. This comparison was completed to determine if a second yet different type of training session altered the hormonal response at selected common time points after of the second training session.

Comparison C = the comparison of the pre and post 07:00 hour time points. This comparison was completed to determine if the hormonal response of subjects the morning following the various trial days was different to pre-trial day values.

The above analyses were completed for all comparisons between the matched control and sequence days, the two control days and the two sequence days.

6.2.5.4. Gene Expression Analysis

Because a relative quantitation method was used to quantify the mRNA content of selected genes (Table 3.4) within the muscle specimens, as opposed to an absolute quantitation method, the two training sequences could not be analysed together in the same repeated measures ANOVA. Consequently, a repeated measures ANOVA with one-within subject factor, time (pre and post W, pre and post C, three hours post the second training session and the 25 hours post the W session) and one-between subject factor, test order was used to determine the effect of the sequence of training over the course of each of the sequence days. Results are presented as mean and standard error however statistical analysis for all genes was carried out on log-transformed data (Keller, Keller, Marshal, & Pedersen, 2003; Mozdziak et al., 1998).

In the event of a violation of the sphericity assumption for any of the dependent variables, the Greenhouse-Geisser correction was used. For all analyses that indicated a significant effect, Bonferroni adjusted multiple pair wise comparisons were completed to locate the source of the difference. An alpha level of .05 was used to indicate a level of significant difference for all ANOVA analysis. All statistical analysis was completed using the Statistical Package for Social Sciences (SPSS version 11.0 for windows, SPSS Inc.).
6.3. Results

6.3.1. Effect of Test Order

The between-subjects factor (test order) was included in all statistical analysis completed however, no effect of test order was found for any of the variables measured (p>.05). To avoid unnecessary repetition of this finding within each variable result, a list of the variables and the corresponding statistical result are provided in Appendix F.

6.3.2. Comparison of the Weight Training Variables

6.3.2.1. Blood Lactate Concentration

No significant difference was found between the WC and CW sequences (p=.237) with both training sequences recording similar pre- and post-training concentrations (Figure 6.2). There was a significant increase in BL concentration from pre- (1.0 ± 0.2 and 1.9 ± 0.6 mmol.L$^{-1}$) to post-training (6.8 ± 2.2 and 6.7 ± 2.8 mmol.L$^{-1}$) for both the WC (p<.001) and CW (p=.002) sequences, respectively. No interaction of training sequence by time (p=.212) was found.

![Figure 6.2. Pre- and post-training BL concentration for the W session of the WC and CW sequences (Mean, error bars represent SD). *: Significantly greater than pre (p<.05)](image-url)
6.3.2.2. Heart Rate

A significantly higher peak HR response for the CW sequence compared to the WC sequence (p=.001) was found across the three exercises. However, post-hoc analysis revealed no significant difference between the sequences for each exercise individually, even though the peak HR response was 6.6, 4.8 and 4.9% higher during the leg-press, -curl and -extension exercises for the CW sequence, respectively (Figure 6.3, top). There was no significant difference between the peak HR response across the exercises for either training sequence (p=.194).

The same analysis was completed for the W session mean HR response and indicated that there was a significant difference between the two training sequences (p=.001). Post-hoc analysis revealed that CW sequence response was significantly higher than the WC sequence, with a 9.8, 9.3 and 8.4% increase in mean HR for the leg-press (p=.017), -curl (p=.017) and -extension (p=.035) exercises, respectively (Figure 6.3, bottom). A significant main effect of exercise (p=.001) was also found. Post-hoc analysis revealed that for both the WC and CW sequences, the leg-press response was significantly less than the leg-curl response (p=.020 and p=.024, respectively) whilst the difference between the leg-press and leg-extension exercises approached significance for the CW sequence (p=.087). There was no significant difference between the leg-curl and -extension for either protocol. There was also no interaction of training sequence by exercise for either peak (p=.886) or mean HR (p=.943).
Figure 6.3. The leg-press, -curl and -extension peak (top) and mean (bottom) HR response during the W session for the WC and CW sequences (Mean, error bars represent SD).

*: Significantly greater than WC (p<.05)
#: Significantly less than leg-curl and -extension (p<.05)
6.3.3. Comparison of the Endurance Cycle Training Variables

6.3.3.1. Blood Lactate Concentration

A significant (p=.010) difference was found between the two training sequences (Figure 6.4). Post-hoc analysis indicated that the subjects had a significantly higher (p<.001) pre-training BL concentration for the WC sequence (2.0 ± 0.5 mmol.L⁻¹) compared to the CW sequence (1.0 ± 0.2 mmol.L⁻¹). However, there was no significant difference between the WC (2.2 ± 1.3 mmol.L⁻¹) and CW (1.7 ± 0.5 mmol.L⁻¹) post-training BL concentrations (p=.281). Even though both training sequences showed increases in BL concentration values from pre- to post-training, there was no main effect of time (p=.149) or interaction of training sequence by time (p=.280).

![Figure 6.4. Pre- and post-training BL concentrations for the C session for the WC and CW sequences (Mean, error bars represent SD).](image)

* : Significantly greater than CW pre (p<.001)
6.3.3.2. Heart Rate

No significant difference was found between the two sequences during the rest and warm-up periods (p=.241) or at any of the time intervals throughout the C session (p=.894). However, the WC sequence did tend to show a slightly higher HR response (approximately 5%) at rest and during the warm-up period and at the final two time periods of the cycling session (Figure 6.5). A significant main effect of period was found for the rest and warm-up periods (p=.001) and the C session (p<.001). Post-hoc analysis indicated that HR increased significantly from rest to the warm-up period and from the warm-up period to the 25-30 minute time period for both protocols (p<.05). Further, the HR response increased significantly over the duration of the 170 minutes of cycling for the WC sequence (p=.012, 8.4%) however no significant effect was found for the CW sequence (p=.320, 5.8%). No interaction of training sequence by period was found (p>.05). The analysis was based on the data from six subjects.

Figure 6.5. Heart rate response for the WC and CW sequences during the rest and warm-up periods and the five sampling time periods of the C session (Mean of six subjects, error bars represent SD).

# : Significantly greater than rest period (p<.05)
* : Significantly greater than 25-30 min time period for the WC sequence (p<.05)
6.3.3.3. Respiratory Responses

The results for the respiratory responses RR, $V_E$, VO$_2$ and RER for the two training sequences over the duration of the endurance cycling training session are based on four subjects due to technical problems experienced with the gas analyser during the training sessions of four of the subjects. In view of the limited subject numbers, statistical analysis was limited to Bonferroni adjusted multiple comparisons across each time interval, which found no significant difference between the two training sequences for the three variables other than VO$_2$ at the 55-60 minute time period ($p=.001$). The lack of statistical power restricted the ability of the analysis to detect significant differences and as such the trends of the results are outlined below.

The average increase in RR for the WC sequence above that of the CW sequence over the duration of the C session was 12.1%, ranging from 13.1% at rest through to 25 and 23.5% at the 115-120 and 145-150 minute time periods, respectively. A similar pattern was also found for $V_E$, with an average increase of 14.7% for the WC sequence over the duration of the training session above that of the CW sequence, ranging from 19% at rest through to 25.3 and 25.8% at the 115-120 and 145-150 minute time periods, respectively. Given the relationship between RR, $V_E$ and VO$_2$, it was expected that there would be an increase in VO$_2$ over the majority of the cycling session for the WC sequence compared to the CW sequence. Whilst this was the case, the impact was not as visually noticeable as for the RR and $V_E$ responses (Figure 6.6a). The average increase in VO$_2$ for the WC sequence above that of the CW sequence was 9.4%, with the greatest difference occurring during the rest interval with a 26.5% difference. Even though the difference reduced substantially during the warm-up to 4.7%, the level of VO$_2$ for the WC sequence above that of the CW sequence increased gradually over the course of the C session with a difference of 7.3%, 8.7% and 8.7% for the final three time periods, respectively. Accordingly, RER for the WC sequence showed a gradual decline from 0.96 at the 25-30 minute period to 0.90 at the 145-150 minute period, an overall reduction in RER of 7% compared to the CW sequence which remained relatively stable over the duration of the C session (Figure 6.6b).

Overall, the respiratory responses indicated a greater aerobic demand for the WC sequence compared to the CW sequence that was accentuated as the duration of the cycling session progressed.
Figure 6.6a. Respiratory responses RR (top), $V_E$ (middle) and $VO_2$ (bottom) for the WC and CW sequences during the rest and warm-up periods and the five sampling time periods of the C session (Mean of four subjects, error bars represent SD).
Figure 6.6b. Respiratory exchange ratio (RER) for the WC and CW sequences during the rest and warm-up periods and the five sampling time periods of the C session (Mean of four subjects, error bars represent SD).
6.3.3.4. Gross and Net Efficiency

The results for GE and NE for the two training sequences over the duration of the C session are based on four subjects for the same reason as outlined for the respiratory responses. Even though subjects experienced a notable reduction in both GE and NE during the WC sequence compared to the CW sequence, Bonferroni adjusted multiple comparisons across each time interval found no significant difference between the two training sequences other than at the 115-120 minute time interval for GE (p=.003). However, the result may reflect the lack of statistical power and hence the ability of the analysis to detect significant differences. Therefore the trends of the results are outlined below.

Both GE and NE showed similar response patterns over the duration of the C session with a reduction in efficiency from the warm-up period to the commencement of the training session and a continual deterioration in efficiency until the last respiratory gas measurement at the 145-150 minute time interval (Figure 6.7). The completion of the C session after the W session reduced GE by approximately 1.77% across the six time intervals, ranging from 1.74% during the warm-up period to 2.29% at the 145-150 minute time period. Similarly, the sequence of completing the training sessions in the order of weight training then cycling reduced NE by approximately 1.64% across the six time periods, ranging from 1.49% during the warm-up period to 2.33 and 2.31% at the 115-120 and 145-150 minute time periods, respectively.
Figure 6.7. Gross (top) and net efficiency (bottom) for the WC and CW sequences during the warm-up period and the five sampling time periods of the C session (Mean of four subjects, error bars represent SD).
6.3.3.5. Rating of Perceived Exertion

The results for RPE for the two training sequences over the duration of the C session are based on seven subjects. Even though there was a notable difference between the two training sequences with subjects reporting a higher level of exertion for the WC sequence compared to the CW sequence (Figure 6.8), the difference was not significant but did approach significance (p=.082). The average increase in RPE across each time period was 10.8%, with the greatest differences occurring during the warm-up (23.1%) and at the 85-90 (9.9%) and 145-150 minute (9.3%) time periods. A significant main effect of period was found (p<.001). Subsequent post-hoc analysis revealed that the RPE response increased significantly from the 25-30 to the 145-150 minute time period for both training sequences (p<.05) indicating an increase in the level of exertion over the 170 minutes of cycling at 60% of VO$_2$ max. No interaction of training sequence by period was found (p=.681).

![Graph showing RPE over time periods for WC and CW sequences](image)

**Figure 6.8.** Rating of perceived exertion for the WC and CW sequences during the warm-up period and the five sampling time periods of the C session (Mean of seven subjects, error bars represent SD).

* : Significantly greater than 25-30 min time period (p<.05)
# : CW sequence, significantly greater than warm-up period (p<.05)
6.3.4. Comparison of Muscle Glycogen Content for the Sequence Days

The results for muscle glycogen content, over the duration for the two training sequences, are based on six subjects due to one subject missing a sample and another having extreme values (greater than two SD from the mean), as determined by the Grubbs method (Zhang, 1986). A significant difference between the two training sequences (p=.028), in addition to a significant main effect of time (p<.001) and an interaction of training sequence by time (p<.001) was found. In order to determine where the differences were between the two training sequences, given that the order of the training sessions was different, it was necessary to do two Bonferroni adjusted post-hoc analyses.

First, to determine if the sequence of training altered glycogen content levels pre and post each similar training session, the two sequences were compared by matching corresponding time points (i.e. comparing pre and post W, pre and post C, 3hr post the second training session and the 25hr post the W training session for both sequences). This comparison revealed a significant difference between the pre (p=.002) and post (p<.001) W session time points, with the CW sequence (268.8 ± 79.6 and 290.8 ± 100.0 mmol.kg⁻¹) showing considerably lower muscle glycogen levels than the WC sequence (604.8 ± 89.9 and 613.5 ± 60.4 mmol.kg⁻¹) for both time points, respectively. Even though the WC sequence (236.8 ± 57.7 mmol.kg⁻¹) showed a lower glycogen concentration for the 3hr post the second training session compared to the CW sequence (260.5 ± 216.1 mmol.kg⁻¹), no significant difference was found for this or the other three remaining time points (p>.05) (Figure 6.9).

Second, the two training sequences were analysed by comparing pre and post W with pre and post C, 3hr post the second training session and the 25hr post the W training session for both sequences, to determine if there was a difference between the two sequences with respect to the initial sequence day sample and also throughout the day irrespective of the type of training completed. The analysis revealed no significant difference between the pre W and pre C session time points for the WC (604.8 ± 89.9 mmol.kg⁻¹) and CW (530.9 ± 119.8 mmol.kg⁻¹) sequence days, respectively. However, a significant difference was found between the WC pre and post C session and the CW pre and post W session (p=.008 and p=.001, respectively) and also between the WC post W and the CW post C (p=.004).
Post-hoc analysis, comparing each time point to the initial pre W or C sample for that sequence, revealed a significant reduction of muscle glycogen levels for the WC sequence immediately post the C session (p=.006) which continued for three hours post-training (p=.007), when the final biopsy was taken for that day. Whilst no significant difference was found between the pre W and 25 hour post W time points (p=.136) for the WC sequence, the results did indicate that glycogen levels had only returned to approximately 73% of pre W levels (604.8 ± 89.9 mmol.kg⁻¹), 25 hours post the W session (443.8 ± 82.6 mmol.kg⁻¹).

Comparison of the time points to the pre C glycogen level for the CW sequence, revealed that the post C (p=.014) and pre W (p=.050) time points were significantly lower and like the WC sequence, the 25 hour post the C session level had only returned to 81% of pre-training levels. The significant interaction of training sequence by time indicated that the influence of the sequence of training on muscle glycogen content depended on the time of sampling.
Figure 6.9. Muscle glycogen content (mmol.kg\(^{-1}\) dry weight) at each of the muscle biopsy time points throughout the WC (top) and CW (bottom) sequence days. The time points have been coded across the two sequence days: dark shaded area = pre W, dark stripes = 1 hr post W, light shaded area = pre C, light stripes = post C, small squares = 18:00 on each sequence day (i.e. 3 hr post the second training session) and bricks = 25 hr post the completion of the W session (Mean of six subjects, error bars represent SD).

*: WC sequence, significantly different to pre W, 1 hr post W, pre C and 25 hr post W (p<.05)
#: CW sequence, significantly different to pre C (p<.05)
†: Significantly less than WC sequence pre W and 1 hr post W (p<.05)
6.3.5. Comparison of the Hormone Responses for the Control and Sequence Days

The results for the testosterone, cortisol and testosterone/cortisol ratio sections are based on the values obtained from six subjects due to missing samples for two of the subjects at one or more time points.

All comparisons between the matched control and sequence days, the two control days and the two sequence days were completed using corresponding time points, that is those time points that were common to both days being compared. For example: the control C and CW sequence days were compared at the common time points pre 07:00, 11:00, 12:00, 14:00, 15:00, 16:00, 18:00 and post 07:00 hour. In addition, some time points were omitted from the comparisons even though they were analysed if they were considered not to affect the interpretation of the hormone response, in order to reduce the number of compared sample points for statistical power purposes. For example: the 11:15 and 11:30 hour time intervals for the pairing of the control day and corresponding sequence day comparisons.

Samples pre and post each training session were not compared for the two training sequences because of the different time intervals for the training protocols (i.e. the W session went for one hour whilst the C session went for three hours). Comparison of different time intervals during the two sequence days could possibly have resulted in differences that reflected diurnal changes and not the protocol itself. Consequently, the comparisons were completed comparing common time points only at the pre 07:00 and then from 15.00 to post 07:00 hour time points.

6.3.6. Testosterone

6.3.6.1. Comparison of Control C and CW sequence days

No significant difference was found between the control C and CW sequence days for comparison A (p=.708) with similar increases in mean testosterone concentration immediately following training of 11 and 8%, respectively. There was also no significant difference between comparisons B (p=.505) and C (p=.653) even though the completion of a W session three hours after the C session did cause a transient increase in the mean testosterone concentration of 14.5% immediately following training compared to pre-training values. In
contrast, the control day showed an 8.4% reduction in testosterone concentration for the same time period. However, one hour after the training session the CW testosterone concentration had returned to a similar level recorded during the control day.

There was however, a significant main effect of time for comparisons A (p<.001) and B (p<.001) but not for comparison C even though it did approach a level of significance (p=.060). Post-hoc analysis indicated that there was a significant reduction in testosterone concentration between the pre 07:00, 14:00 (p=.043) and 15:00 (p=.037) hour time points for the control day (Figure 6.10). In addition, a significant difference was also found between the 11:00, 12:00 (p=.021) and 14:00 (p=.008) hour time points. A significant difference was found between the pre 07:00 and 16:00 hour time points (p=.037) as well as between the 11:00 hour time point and the start of the W session at 14:00 hours (p=.024) for the CW sequence day. No interaction of training sequence by time was found for the three comparisons.

![Testosterone Response](image)

**Figure 6.10.** Plasma testosterone response over the duration of the day for the control C (Con. C) and CW sequence (Mean of six subjects, error bars represent SD).

* : Con. C, significantly less than pre 07:00 hour (p<.05)
# : Con. C, significantly less than 11:00 hour (p<.05)
† : CW sequence, significantly less than pre 07:00 hour (p<.05)
6.3.6.2. Comparison of Control W and WC sequence days

No significant difference was found between the control W and WC sequence days for comparison A (p=.861) with similar reductions in mean testosterone concentration immediately following training of 14 and 10%, respectively (Figure 6.11). There was also no significant difference between comparisons B (p=.137) and C (p=.456). Similar to the control C and CW comparison above, the completion of a C session three hours after the W session also caused a transient increase in the mean testosterone concentration immediately following training compared to pre-training values by 38%. The control day also showed an increase in testosterone concentration for the same time period but not to the same extent showing only a 25% increase. One hour after the training session the WC testosterone concentration (5.25 ± 2.88 ng.mL^{-1}) had returned to near the level recorded during the control day (4.92 ± 1.63 ng.mL^{-1}).

A significant main effect of time was found for comparisons A (p<.001) and B (p<.001) but not for comparison C (p=.798). Post-hoc analysis indicated that the was a significant reduction in testosterone concentration from the pre 07:00 to the 10:00 hour time points for the control day (p=.031). There was also a significant reduction for the same time points (p=.006) for the WC sequence day, in addition to a significant difference between the immediately post-training time point at 15:00 hour and the 16:00 (p=.045) and 18:00 (p=.010) hour time points, respectively. No interaction of training sequence by time was found for the three comparisons.

6.3.6.3. Comparison of Control C and Control W days

Compared to the pre 07:00 hour concentration, the control C session resulted in a 12% increase in testosterone immediately post-training compared to the control W session, which showed a 14% reduction. However, whilst the plasma testosterone responses immediately post-training were different, comparison of the control C and W days revealed no significant difference between the days for comparison A (p=.110) or B (p=.175) but comparison C did approach a level of significance (p=.098). A significant main effect of time was found for comparison A (p=.001) and B (p<.001) but not C (p=.083). Post-hoc analysis revealed that, compared to the pre 07:00 hour time point, there was a significant reduction in testosterone concentration at the 12:00 and 15:00 hour time points for the control W (p=.013) and C (p=.037) days, respectively (Figure 6.12).
Figure 6.11. Plasma testosterone response over the duration of the day for the control W (Con. W) and WC sequence (Mean of six subjects, error bars represent SD).

* : Significantly less than pre 07:00 (p<.05)
# : WC sequence, significantly less than 15:00 (p<.05)

Figure 6.12. Plasma testosterone response over the duration of the day for the control W (Con. W) and control C (Con. C) (Mean of six subjects, error bars represent SD).

* : Control W, significantly less than pre 07:00 hour (p<.05)
# : Control C, significantly less than pre 07:00 hour (p<.05)
6.3.6.4. Comparison of CW and WC sequence days

No significant difference was found between the CW and WC sequence days for comparison A (p=.370) with similar mean testosterone concentrations at pre 07:00 (6.77 ± 1.30 and 5.93 ± 1.35 ng.mL⁻¹) and 12:00 hour (5.35 ± 1.22 and 5.05 ± 1.84 ng.mL⁻¹), respectively. There was also no significant difference between comparisons B (p=.326) and C (p=.837) even though the completion of a C session three hours after the W session caused a 38% temporal increase in the mean testosterone concentration immediately following training compared to the W session response of 14% for the CW sequence. Whilst not significant, the WC mean testosterone concentration remained elevated above that of the CW by 29, 60, 21 and 18% for the four respective time points immediately following cessation of the C session before returning to similar values recorded during the CW sequence at the 18:00 hour (Figure 6.13).

There was a significant main effect of time for comparisons A (p<.010) and B (p<.001) but not for comparison C (p=.626). Post-hoc analysis indicated that there was a significant reduction in testosterone concentration between the pre 07:00 and 12:00 hour time points for both the WC (p=.021) and CW (p=.020) sequences. The CW sequence also showed a significant reduction between the 15:00 hour time point and the time point 30 minutes later at 15:30 hour (p=.021). The testosterone concentration was significantly less at 18:00 hour compared to the 15:00 and 15:15 hour time points (p=.026 and p=.020, respectively) for the WC sequence. No interaction of training sequence by time was found for the comparisons A (p=.277) or C (p=.172) but there was a significant interaction for comparison B (p<.001).
6.3.7. Cortisol

6.3.7.1. Comparison of Control C and CW sequence days

Similar to testosterone, no significant difference was found between the control C and CW sequence days for comparison A (p=.065), B (p=.158) or C (p=.245). Though not significant, the completion of the W session three hours after the C session did cause a transient increase of 9% in the mean cortisol concentration immediately following training compared to pre-training values (Figure 6.14). In contrast, the control day showed an 8% reduction for the same time period. One hour after the training session the CW mean cortisol concentration was still elevated above that of the control day but returned to a similar concentration by three hours post-training (18:00 hour).

A significant main effect of time was found for comparisons A (p=.03) and B (p=.002) but not for C (p=.923). Post-hoc analysis revealed that there was a significant reduction in cortisol concentration from the pre 07:00 to the 16:00 (p=.038) hour time point for the CW sequence. A significant reduction was also found between the 11:00 and 12:00 (p=.038) hour time points for the CW sequence. No significant differences were found for the control day however,
there were large differences between subjects in response to the control and sequence day training protocols. No interaction of training sequence by time was found for any of the comparisons.

![Graph showing cortisol response over the day](image)

**Figure 6.14.** Plasma cortisol response over the duration of the day for the control C only (Con. C) and CW sequence (Mean of six subjects, error bars represent SD).

* : CW sequence, significantly less than 11:00 hour (p<.05)
# : CW sequence, significantly less than pre 07:00 hour (p<.05)

### 6.3.7.2. Comparison of Control W and WC sequence days

No significant difference was found between the control W and WC sequence day up to and including the commencement time point (12:00 hour) of the C session time, that is comparison A (p=.109). However, following the C session a significant difference was found between the two trial days (p=.001). Post-hoc analysis indicated that the WC sequence cortisol response (17.71 ± 9.42 and 10.61 ± 3.36 µg.dL⁻¹) was significantly greater than the control day response (6.43 ± 1.42 and 6.71 ± 2.57 µg.dL⁻¹) at the 15:00 (p=.028) and 16:00 (p=.046) hour time points, respectively (Figure 6.15). The WC cortisol concentration (7.60 ± 3.92 µg.dL⁻¹) was still elevated above that of the control day (4.25 ± 1.57 µg.dL⁻¹) three hours after the completion of the C session however, had returned to control day levels the
following morning (07:00 hour). No significant difference was found for comparison C (p=.996).

A significant main effect of time was found for comparisons A (p=.003) and B (p=.005) but not for comparison C (p=.840). Post-hoc analysis indicated that there was a significant reduction in cortisol concentration from the pre 07:00 to the 12:00 (p=.046), 16:00 (p=.001) and 18:00 (p=.006) hour time points for the control day (p=.046). Even though the completion of the C session three hours after the W session caused a 157% increase in the mean cortisol concentration (17.71 ± 9.42 µg.dL⁻¹) immediately following training compared to pre-training levels (6.90 ± 2.39 µg.dL⁻¹), no significant differences were found between the time points for the WC sequence. As was the case with the control C and CW sequence comparison, there were large variations between subjects in response to the control and sequence day training protocols, evident by the large SD for the 15:00 hour time point for the WC sequence. No interaction of training sequence by time was found for the three comparisons.

![Figure 6.15.](image)

**Figure 6.15.** Plasma cortisol response over the duration of the day for the control W (Con. W) and WC sequence (Mean of six subjects, error bars represent SD).

* : Control W, significantly less than pre 07:00 hour (p<.05)

# : WC sequence significantly greater than control W (p<.05)
6.3.7.3. Comparison of Control C and Control W days

The C session caused an increase in mean cortisol concentration of 15% immediately following the training session compared to the pre 07:00 hour time point (Figure 6.16). In contrast, the W training session caused an 18% decrease in mean cortisol concentration immediately following the training session compared to the pre 07:00 hour time point. Whilst there was a difference in the response patterns immediately following training for the two control days, no significant difference was found for comparison A (p=.198), B (p=.631) or C (p=.284), indicating similar response patterns over the course of the day from the 12:00 hour onwards. A significant main effect of time was found for comparison A (p=.011) and B (p=.003). Post-hoc comparisons indicated a significant reduction in cortisol concentration from the pre 07:00 to the 12:00 (p=.025), 16:00 (p=.001) and 18:00 (p=.006) hour time points for the control W day (p=.046). However, no significant differences were found for the control C day.

![Graph showing plasma cortisol response over the duration of the day for control W (Con. W) and control C (Con. C).](image)

* : Control W, significantly less than pre 07:00 hour (p<.05)
6.3.7.4. Comparison of CW and WC sequence days

A significant difference was found between the CW and WC sequence days for both comparison B (p=.048) and C (p=.016) but not comparison A (p=.125). Post-hoc analysis revealed that the cortisol response 15 minutes immediately post the C session for the WC sequence (14.07 ± 5.75 µg.dL^{-1}) was significantly (p=.041) greater than the CW sequence (8.56 ± 3.10 µg.dL^{-1}) at the same time point (Figure 6.17). The 15:00, 15:30 and 16:00 hour time points all approached a level of significance (p<.066). The WC sequence mean cortisol response was still elevated above that of the CW sequence at 18:00 hour by 33% but the difference was not significant (p=.577). Even though a significant difference was found for comparison C, post-hoc analysis did not reveal a significant difference between the two sequences for either time point.

A significant main effect of time was found for comparison A (p=.010) and B (p=.003) but not C (p=.356). Post-hoc analysis revealed that the WC and CW sequences both showed significant reductions from the pre 07:00 to 12:00 hour time point (p=.012 and p=.039, respectively). However, no significant changes were found for the remaining time points compared to the pre 07:00 hour time point, a fact that could be attributed to the notably different responses of the subjects to the training protocols.
6.3.8. Testosterone/Cortisol Ratio

6.3.8.1. Comparison of Control C and CW sequence days

No significant difference was found between the control C and CW sequence days for comparison A (p=.354), B (p=.748) or C (p=.391) with similar patterns of change over the course of the day up to the time corresponding to finish of the W session (15:00 hour). Whilst no significant difference was found between the trial days post the W session (comparison B), there was a difference in the timing of the changes as evident by the peak in the ratio for the control day at 16:00 hour compared to the CW sequence which peaked at 18:00 hour (Figure 6.18). This difference was not found the following morning at the post 07:00 hour time point with similar ratios for the two sequences.

A significant main effect of time was found for comparison B (p=.004) however, post-hoc analysis did not reveal a significant difference between any of the time points. No main effect
of time was found for comparison A (p=.360) or C (p=.315). No interaction of training sequence by time was found for any of the comparisons.

![Graph](image)

**Figure 6.18.** Testosterone/cortisol (T/C) ratio over the duration of the day for the control C (Con. C) and CW sequence (Mean of six subjects, error bars represent SD).

### 6.3.8.2. Comparison of Control W and WC sequence days

No significant difference was found between the control W and WC sequence days up to and including the commencement of the C session time point (p=.629). However, comparison of the time points post the C session revealed a significant difference (p=.032) between the trial days (Figure 6.19). Post-hoc analysis did not identify any significant differences between the trials but it is worth pointing out that the 15:00 and 16:00 hour time points did approach a level of significance (p=.067 and p=.057, respectively). There was no significant difference between the trial days for comparison C (p=.229).

A significant main effect of time was found for comparison B (p<.003) only. Subsequent post-hoc analysis did not identify any significant differences between time points for either trial day (p>.05), which could be attributed to the large individual variances between subjects for plasma testosterone and cortisol, as outlined above.
6.3.8.3. Comparison of Control C and Control W days

No significant difference was found between the two control days for comparison A (p=.872), B (p=.277) or C (p=.217), with a similar response pattern across the majority of the time points (Figure 6.20). A significant main effect of time was found for comparison B (p=.027) but not A (p=.621) or C (p=.431). Post-hoc analysis revealed a significant increase in the testosterone/cortisol ratio from the pre 07:00 to 16:00 (p=.050) hour time point for the control W day. Even though the control W day showed a large rise in the testosterone/cortisol ratio at the 18:00 hour time point it was not significantly different to other time points because of the large variation in subject responses at this time, as outlined in the individual testosterone and cortisol comparisons above. No significant differences were found between the time points for the control C day.
Figure 6.20. Testosterone/cortisol (T/C) ratio over the duration of the day for the control W (Con. W) and control C (Con. C) (Mean of six subjects, error bars represent SD).

* : Control W, significantly greater than pre 07:00 hour (p<.05)

6.3.8.4. Comparison of CW and WC sequence days

Comparisons A (p=.360), B (p=.426) and C (p=.153) did not reveal any significant difference between the two control days even though there was a difference in the testosterone/cortisol ratio response at the 12.00 and 18:00 hour time points for the two sequences (Figure 6.21). A significant main effect of time was found for comparisons A (p<.040) and B (p<.001) but not for comparison C (p=.983). Post-hoc analysis indicated that there was a significant increase in the testosterone/cortisol ratio between the pre 07:00 and 12:00 hour time points for the WC sequence (p=.047). No significant differences were found between the remaining time points for either sequence. There was also no interaction of training sequence by time for any of the comparisons.
6.3.9. Gene Expression

The muscle mRNA content of the selected genes at the sampling time points, over the duration for the two training sequences, are based on seven subjects. A number of the genes showed a significant main effect of time for one or both of the training sequences, as outlined below. It should be noted that some of the genes examined showed distinct changes over the duration of the sequence days but statistical analysis did not show a significant effect. This can be attributed in part to the distribution of the values hence the reason for the statistical analysis being completed using log transformed data (Keller et al., 2003; Mozdziak et al., 1998).

6.3.9.1. Expression of genes associated with muscle growth

Myogenin – a significant main effect of time was found for the CW sequence (p<.001) but not the WC sequence however, it did approach a level of significance (p=.079). Post-hoc analysis indicated that following the W session in the CW sequence there were significant increases (~5-6 fold) in mRNA levels at the 1, 3 and 25 hour post W session time points.
relative to the pre and post C time points (Figure 6.22, top). The pre W time point approached significance (p=.059), recording a similar magnitude of change.

**MyoD** – a significant main effect of time was found for both the WC (p=.017) and CW (p<.001) sequences however, post-hoc analysis only revealed a significant change (~5 fold) for the CW sequence for the 25 hour post W (p=.039) compared to the pre C time point (Figure 6.22, bottom). This time point was also found to be significantly greater than the post C (p=.029) and 1 (p=.021) and 3 hour post W (p=.001) time points.

**MHCIIa** – no significant changes were found for the WC or CW sequences (Figure 6.23, top) even though a significant main effect of time was found for the CW sequence (p.011). Whilst some of the time points showed notable mean fold changes due to the results of a few subjects, the majority of time points showed little or modest changes (< 4 fold).

**MHCIIx** – no results were achieved for this gene due to problems associated with the RNA analysis stemming from the prior optimisation process.

**IGF1 & 2** – no significant main effect of time was found for the WC sequence (p=.825) with most time points showing little change compared to pre W (Figure 6.26, bottom). A significant main effect of time was found for the CW sequence (p=.038), however subsequent post-hoc analysis did not reveal any significant difference between the time points, with the only time point showing any real change, yet it be small (~2 fold) was the pre W time point.

### 6.3.9.2. Expression of genes associated with muscle metabolism

**PDK4** – a significant main effect of time was found for both the WC (p=.012) and CW (p<.001) sequences. Post-hoc analysis revealed that the change in mRNA content from the pre C to the 3 hour post C time point (~28 fold) was significant (p=.024) as well as the change from the 3 hour post C to the 25 hour post W (~20 fold, p=.009) for the WC sequence (Figure 6.24, top). Large mean changes of between 10 and 22 fold were found for some of the other
time points but these were due to the influence of a few subjects who showed considerable change following the respective training sessions. No significant changes were found across any of the time points for the CW sequence however, a number of them did approach significance, namely the pre W (p=.060) and 1 (p=.055) and 3 hour post W (p=.068) time points, showing large fold changes of between 8 and 18.

PGC1 – a significant main effect of time was found for both the WC and CW sequences (p<.001). The post C (~7 fold, p=.021) and 3 hour post C (~27 fold, p=.006) time points showed significant change compared to the pre W time point for the WC sequence (Figure 6.24, bottom). The 3 hour post C time point was also significantly greater than the pre (p=.004) and post C (p=.008) time points. Following the peak at the 3 hour post C time point, the mRNA content significantly reduced to pre W levels at the 25 hour post W time point (p=.015). In contrast, the pre W and 1 hour post W time points for the WC sequence showed a significant fold increase compared to pre C (~ 13 fold, p=.002 and ~7 fold, p=.001, respectively). These time points were also significantly greater (p<.05) than the 25 hour post W time point which had returned to near pre C levels.

HKII - a significant main effect of time was found for both the WC and CW sequences (p<.001). A significant large change in mRNA content (~11 fold, p=.004 and ~10 fold, p=.026) was found 3 and 25 hours post the C and W sessions for the WC sequence, respectively (Figure 6.25, top). The 3 hour post W level was also significantly greater than the 1 hour post W time point (p=.05). All time points other than 25 hour post W showed significant mean changes between ~3-6.5 fold for the CW sequence, with the greatest being at the pre W time point (~6.5 fold).

LPL – even though a significant main effect of time was found for both the WC (p=.019) and CW (p=.019) sequences, post-hoc analysis did not reveal any significant difference between any of the time points (Figure 6.25, bottom). Compared to the pre W and pre C time points, the WC sequence showed small to moderate changes (~2-4 fold), whilst the CW sequence showed very little change, respectively.

GYS – no significant main effect of time was found for the WC (p=.433) or CW (p=.370) sequence, with both sequences showing no or only small changes (< ~2 fold) over the six time points (Figure 6.26, top).
**UCP3** – a significant main effect of time was found for both the WC (p=.024) and CW (p=.001) sequences however, no significant changes were found for any of the time points for either sequence in the post-hoc analysis. The WC showed very little change (<~2 fold) across the time points whilst a moderate change (~4 fold) from the pre C to pre W time points was found for the CW sequence (Figure 6.27), but the difference was not significant (p>.05).

No significant main effect of time was found for the WC or CW sequence for the three remaining metabolic genes, LDH-M (p=.308 and p=.528), LDH-H (p=.162 and p=.312) and CPT1 (p=.179 and p=.304), respectively. Furthermore, no or very little change (<~2 fold) was found for all three genes across the majority of the time points for both sequences. The few points that did show larger changes (~4 fold) than the other points were due to the effect of one or two subjects.
Figure 6.22. Myogenin (top) and MyoD (bottom) mRNA content in the vastus lateralis prior to and following the W and C sessions for the WC and CW sequence days (Mean of seven subjects, error bars represent standard error).

* : CW sequence, significantly greater than pre C (p<.05)
# : CW sequence, significantly greater than post C (p<.05)
† : CW sequence, significantly greater than pre and post C and 1 and 3 post W (p<.05)
Figure 6.23. MHC I (top) and MHC IIa (bottom) mRNA content in the vastus lateralis prior to and following the W and C sessions for the WC and CW sequence days (Mean of seven subjects, error bars represent standard error).
Figure 6.24. PDK4 (top) and PGC1 (bottom) mRNA content in the vastus lateralis prior to and following the W and C sessions for the WC and CW sequence days (Mean of seven subjects, error bars represent standard error).

* : WC sequence, significantly greater than pre W (p<.05)
# : WC sequence, significantly less than 3 post W (p<.05)
† : WC sequence, significantly greater than pre C and post C (p<.05)
x : CW sequence, significantly greater than pre C (p<.05)
o : CW sequence, significantly less than pre W and 1 post W (p<.05)
Figure 6.25. HKII (top) and LPL (bottom) mRNA content in the vastus lateralis prior to and following the W and C training sessions for the WC and CW sequence days (Mean of seven subjects, error bars represent standard error).

* : WC sequence, significantly greater than pre W (p<.05)
# : WC sequence, significantly greater than 1 post W (p<.05)
† : CW sequence, significantly greater than pre C (p<.05)
Figure 6.26. GYS (top) and IGF1-2 (bottom) mRNA content in the vastus lateralis prior to and following the W and C training sessions for the WC and CW sequence days (Mean of seven subjects, error bars represent standard error).
Figure 6.27. UCP3 mRNA content in the vastus lateralis prior to and following the W and C training sessions for the WC and CW sequence days (Mean of seven subjects, error bars represent standard error).

6.3.10. Summary of Results

In summary,

- Higher BL and HR responses were found in those sessions completed second in the training sequences. A greater increase in respiratory responses and RPE along with a greater reduction in GE, NE and RER were found during the C session of the WC sequence compared to the CW sequence. Furthermore, the respiratory responses of the WC sequence progressively increased at a faster rate over the last 60-90 minutes of the 170-minute C session, compared to the relatively stable responses for the CW sequence.

- A significant difference in muscle glycogen levels was found between the two training sequences. The CW sequence showed considerably lower muscle glycogen levels than the WC sequence at the pre and post W time points, due to a
significant reduction produced by the prior C session of ~70% from pre- to post-training. No significant difference was found between the remaining time points.

- Both sequence days showed temporal increases in testosterone and cortisol following the second training sessions compared to the control days. Even though no significant difference was found between the two training sequences for testosterone or cortisol, the WC sequence showed a greater response post the second training session than the CW sequence. The WC sequence also produced a reduced T/C ratio compared to the CW sequence in the hours following the second training session.

- The muscle regulatory factor genes, MyoD and myogenin were affected by the sequence of training, responding more to the CW sequence than the WC sequence, especially at the 25 hour post W time point where significant ~5-6 fold increases in mRNA content were found. In contrast, the genes PDK4, HKII, PGC1 and LPL, which are associated with metabolic functions, responded more to the WC sequence than the CW sequence. The remaining genes associated with muscle metabolic function did not respond more to one training sequence than another.
6.4. Discussion

6.4.1. Introduction

A number of mechanisms have been proposed by investigators to explain why the adaptations following concurrent training sessions are impeded compared to those of single mode strength (Costill et al., 1979; Kraemer et al., 1988) and endurance (Holloszy & Coyle, 1984; Kraemer, 2000a; Terjung, 1995) training regimes. These include neuromuscular fatigue, muscle damage, muscle glycogen depletion, alterations in the concentrations of various anabolic/catabolic hormones, differences in the organisation of neuromuscular recruitment patterns and changes in muscle fibre transformation and hypertrophy processes (Chromiak & Mulvaney, 1990; Leveritt et al., 1999). However, because there has been limited attention given to these mechanisms, the role that muscle glycogen depletion, changes in hormone concentrations or possible alterations in protein synthesis due to changes in skeletal muscle gene expression have on concurrent training responses are still unknown. Furthermore, whether the sequence in which the training sessions are completed is an important factor in determining the responses of these mechanisms.

The aim of this study was to investigate whether the sequence of completing strength and endurance training sessions on the same day altered hormonal responses and muscle gene expression following the different types of training as well as muscle glycogen utilisation during and following recovery from exercise. The major findings from this study were that the training sessions completed second in the training sequences (WC and CW) were performed at a higher physiological stress compared to when the same sessions were completed first. Further, that the higher physiological cost of the second session influenced the choice of substrate utilisation during exercise. The increased physiological stress and the reduction of muscle glycogen stores post-training were paralleled by the hormonal responses with a marked increase in testosterone and cortisol for those training sessions completed second in the training sequence. In addition, the sequence of completing concurrent training sessions was found to affect the expression of some of genes associated with muscle growth and metabolism, in conjunction with the combination of the increased physiological stress and the reduction of muscle glycogen stores, respectively.


6.4.2. The Effect of the Sequence of Concurrent Training Sessions on the Physiological Responses of Individual Training Sessions

Previous research has shown that during dynamic strength training exercise heart rate can increase dramatically above resting values (Fleck, 1988; Stone, Fleck, Triplett, & Kraemer, 1991), with values as high as 170 b.min\(^{-1}\) for a two-legged leg-press at 95% of 1 RM to failure being reported (MacDougall, Tuxen, Sale, Moroz, & Sutton, 1985). The peak HR values found for the W session of the WC sequence in the present study were not as high as those reported by MacDougall et al (1985). However, they did indicate, along with the mean response, that HR can increase substantially above resting levels.

Whilst the acute cardiovascular responses to strength training have not been extensively researched (Fleck, 1992), there has been even less research conducted on the effects of prior endurance or strength training on the acute cardiovascular responses during a strength training session. However, given that elevated physiological responses during a second training session in one day have been reported previously for endurance exercise bouts (Ronsen et al., 2001), it is conceivable that a W training session completed following cycling would produce a similar effect if the subjects had not fully recovered. The current findings support this suggestion, with a significant increase in mean HR as well as the notable increase in peak HR for all exercises of the W session of the CW sequence compared to the WC sequence. This indicated a greater physiological stress during the W session of the CW sequence and incomplete recovery from the prior C session. The responses for the hormones testosterone and cortisol, addressed later in this discussion, also suggested a greater level of stress during the W session of the CW sequence with increased testosterone and cortisol concentrations post-training compared to the control day and WC sequence responses of the present study.

Apart from the factors that are known to cause prolonged physiological disturbances of resting metabolism (residual effects of elevated hormones, shift in substrate source and repair of damaged tissue) which may have contributed to the elevated HR responses during the respective leg-strength training exercises of the W session for the CW sequence, the responses could also be due to an impedance of blood flow during the strength training exercises. This impedance due to an increase in muscle tension, the result of a greater activation of leg muscle mass in response to muscle fatigue (Fleck, 1988; Fleck et al., 1989; St Clair Gibson et al., 2001). In association with an increase in muscle mass activation of the exercising limbs,
an increased intrathoracic pressure from a greater level of exertion by the subjects could have also contributed to the finding (Brooks et al., 2000; Fleck, 1988). The increased cardiovascular stress during the W session of the CW sequence indicates that a longer recovery period than three hours between training sessions was needed to enable complete recovery from the effects of the prior C session.

No statistical analysis could be completed for the respiratory or efficiency variables for the C training sessions because of the small subject numbers. However, the results of the WC sequence demonstrated a trend towards a greater physiological stress during the C session compared to the CW sequence. Prior to the commencement of the C session for the WC sequence, the resting results indicated an elevated state, as the cardiorespiratory responses, HR (5%), RR (13%), $V_{E}$ (19%) and VO$_2$ (26.5%) all showed markedly higher values than the CW sequence. The elevated physiological values for the WC continued into the exercise period of the session, progressively increasing at a faster rate over the last 60-90 minutes of the 170-minute C session for the WC sequence, compared to the relatively stable responses for the CW sequence. This response pattern, along with the notable difference in GE and NE between the two sequences, suggests an element of muscular fatigue resulting in increased energy expenditure. The factors that may have contributed to the differences between the two training sequences, that is the type of strength training protocol utilised and the duration of the C session, are discussed in more detail in the General Discussion.

In light of the level of muscle fatigue sustained during the W session for the WC sequence in the present study, it is conceivable that the increased energy expenditure for the exercising component of the C session is probably due to the recruitment of additional muscle fibres. During prolonged exercise, muscle fibre recruitment is orientated towards the use of slow twitch and fast twitch oxidative fibres. However, as the preferred fibres become fatigued and glycogen levels depleted, there is increased recruitment of fast twitch glycolytic fibres (Gollnick et al., 1974; Miura, Kitagawa, & Ishiko, 1997; Vollestad et al., 1984). Because of the high level of muscle glycogen utilisation during the C session along with the muscle fatigue already sustained from the prior W session, the amount of muscle mass recruited and the level of muscle fatigue sustained during the three hours of cycling would have more than likely been compounded for the WC sequence. The significantly greater BL concentration prior to the commencement of the C session for the WC session and the marginally higher but non-significant post-training concentration support this suggestion. However, because of the
level of muscle glycogen depletion for the C session, the true contribution of anaerobic metabolism to force production is disguised due to the probable use of BL as a fuel source (Costill et al., 1988) and as such the difference in BL concentration between the training sequences may have been reduced. The possible use of BL as a fuel may also explain why the BL concentration following the two W training sessions did not show any significant difference. This point is addressed in more detail in a comparison of Study 2 and 3 in the General Discussion.

In view of the increased level of muscle fatigue sustained prior to the C session and the increased amount of exercising muscle mass during the C session, it was no surprise to find that the percentage contribution of substrate being utilised during the session for the WC sequence altered over the course of the 170 minutes, as an increase in exercising muscle mass means that there would be an increased rate of muscle glycogen depletion (Gollnick, Armstrong, Sembrowich et al., 1973; Maclaren et al., 1989). Furthermore, during prolonged endurance exercise there is typically a shift in substrate utilisation from carbohydrate to fat (Hultman & Greenhaff, 1992; Newsholme, Blomstrand, McAndrew, & Parry-Billings, 1992) resulting in an increase in oxygen consumption to produce the same amount of ATP due to a reduction in phosphorylation-coupling efficiency (Bahr et al., 1991). The current findings support the above notion, for the WC sequence showed a similar effect, as there was a progressive rise in VO\textsubscript{2} over the duration of the C session, which coincided with a steady decline in RER, indicating a shift away from carbohydrate metabolism. In contrast, the C session for the CW sequence showed very little change over the 170 minutes either for the respiratory responses or RER, suggesting a lesser reliance on lipid oxidation. Even though there was no significant difference in muscle glycogen content post the C session for the two training sequences, the respiratory and RER responses suggest that this may be related to the level or rate of muscle glycogen depletion. This pattern of change may have also been influenced by the hormonal responses of the C session for the WC sequence, as an increase in the cortisol response compared to the CW sequence was found following the C session. This scenario is discussed in more detail in muscle glycogen section below.

### 6.4.3. Effect of the Sequence of Training on Muscle Glycogen Content

One of the sources attributed to residual fatigue during concurrent training regimes is muscle glycogen depletion. This source of muscle fatigue has been proposed because of the well
documented evidence that indicates that an acute bout of endurance exercise can considerably reduce muscle glycogen stores (Costill, Bowers et al., 1971; Costill, Sparks et al., 1971; Gollnick, Armstrong, Saubert et al., 1973; Saltin & Hermansen, 1967; Sherman et al., 1984; Tarnopolsky et al., 1990). Because of the depletion of muscle glycogen stores following endurance exercise, the performance of strength training using the same muscle groups in the period of time before muscle glycogen levels have been replenished might restrict the performance capabilities of the following activity, either in the level of force generation (exercise intensity) or time duration of the exercise (Grisdale et al., 1990). Whilst muscle glycogen depletion has been questioned as a source of muscle fatigue because of the findings of some concurrent training (Dudley & Djamil, 1985; Hickson, 1980) and individual endurance studies (Sahlin & Seger, 1995; Sherman et al., 1984), it has not been previously investigated in a concurrent training regime. Therefore the influence that muscle glycogen content has on the performance of concurrent training sessions is unknown and hence the inclusion in this study.

The majority of studies that have investigated substrate utilisation during strength training have reported depleted muscle glycogen stores ranging from 12 to 38% immediately post-exercise (Pascoe et al., 1993; Robergs et al., 1991; Tesch et al., 1986). In the present study, the fact that the W session for both sequences did not result in a reduction in muscle glycogen stores as reported previously maybe due to the design of the strength training protocol, the time of muscle sampling, as well as the provision of a meal one-hour prior to the commencement of training. Previous studies (Pascoe et al., 1993; Robergs et al., 1991; Tesch et al., 1986) that have reported a reduction in muscle glycogen content following strength training have used low-moderate intensity exercise (~35-70% of 1 RM) with short duration rest periods (30-120 seconds) resulting in a greater exercise stress as evident by high levels of lactate produced (ranging from ~6-15 mmol.L⁻¹). In contrast, the current study used a high-intensity load (85% of 1 RM) with three-minute rest periods, which reduced the duration of loading during sets (< 30 seconds) and allowed a longer recovery between sets and therefore possibly reducing the level of glycogen depletion post-training. In addition, previous studies (Pascoe et al., 1993; Robergs et al., 1991; Tesch et al., 1986) have also taken muscle specimens immediately post-training whereas one-hour post-training sampling was used in this study. In conjunction with the provision of a meal one-hour prior to training, this may have enabled the replenishment of muscle glycogen stores prior to sampling. The use of blood lactate by the muscles to replenish glycogen stores (Bangsbo, Graham, Kiens, & Saltin, 1992)
in the time between the completion of the W session and the time of the muscle sample may have also aided the recovery process, particularly in the case of the CW sequence where muscle glycogen levels were significantly lower than the WC sequence prior to the W session.

The present results of the C session of the CW and WC sequences were consistent with previous reports that there is a pronounced reduction of muscle glycogen stores following prolonged endurance activity (Costill, Bowers et al., 1971; Costill, Sparks et al., 1971; Gollnick, Armstrong, Saubert et al., 1973; Saltin & Hermansen, 1967; Sherman et al., 1984; Tarnopolsky et al., 1990). However, the interesting finding of the present study was that the completion of the W session prior to the C session in the WC sequence did not significantly alter the level of muscle glycogen immediately post-exercise, with similar values found for both training sequences. This finding suggests that the rate of utilisation of muscle glycogen was similar between the training sequences. However, there was a difference in the RER for the C sessions. The notable reduction in the RER for the C session of the WC sequence compared to the CW sequence indicates a shift in substrate utilisation from carbohydrate metabolism towards lipid oxidation (Costill et al., 1988). This suggests that the choice of substrate was in response to the rate of muscle glycogen decline and/or to provide a muscle glycogen sparing response. The notable increase in cortisol for the WC sequence compared to the corresponding control day as well as the CW sequence, which showed very little change over the duration of the C session, supports the possibility of a glycogen sparing response, as cortisol has been found to be associated with the mobilisation of triglycerides by lipolysis in response to low glycogen levels (Chattoraj & Watts, 1986; Galbo, 1992; Kraemer, 2000b).

A similar muscle glycogen sparing response to that proposed above was also reported previously by Costill et al (1988) during three successive days of endurance running. It was found that there was an increased mobilisation of free fatty acids at rest and during exercise on the second and third days, which coincided with progressively lower RER values. Furthermore, they calculated that fat utilisation increased progressively from 15.2 grams on day one to 38.4 and 46.4 grams on days two and three, respectively, as the level of muscle glycogen pre-exercise declined on each subsequent day.

The significant reduction in muscle glycogen content following the C sessions of the present study confirmed that the ingestion of carbohydrates during exercise does not stop the depletion of muscle glycogen stores but may assist in the extending the time to fatigue.
Whilst the subjects were able to complete the C session for both training sequences, the level of muscle glycogen depletion (~70% of pre-exercise levels) was substantial and an indication of the importance of carbohydrate feeding during endurance exercise as well as immediately upon completion, particularly if subsequent training sessions are to be completed within close proximity. Furthermore, the level of muscle glycogen depletion following the training sessions and sequences also highlighted the importance of investigating a simple procedure for measuring glycogen replenishment post-exercise.

Similar to Costill et al (1988) who reported that diminished muscle glycogen levels did not impair strength following 10 successive days of intense swimming, the present finding of reduced muscle glycogen levels from the C session for the CW sequence did not prevent the completion of the W session. Apart from a possible increase in the contribution of fat utilisation to energy supply, the current finding and that of Costill et al (1988) may be due to the level of depletion being less than the level required to impair force-generating capacity and therefore prevent subjects completing the exercises. This suggestion is supported by the findings of Jacobs (1981) and his colleagues (Jacobs et al., 1981) who investigated the relationship between muscle glycogen levels and quadriceps strength and reported that muscle strength was only reduced when the glycogen content of the muscle was below a certain level.

The other possible explanations may have been that the level of glycogen depletion was selective to specific types of fibres (i.e. slow twitch fibres) or that sufficient fast twitch fibres had recovered following the endurance cycling to enable the completion of the weight training session. Previous research has shown that following prolonged sub-maximal endurance exercise there is a significant depletion of muscle glycogen stores but mainly in slow twitch fibres (Gollnick et al., 1974; Jacobs et al., 1981) and if exercise duration was to continue that there is an increased recruitment of fast twitch fibres to compensate (Vøllestad et al., 1984). In view of the moderate intensity of the C session (60% of VO₂ max) of the present study, which would have targeted slow twitch fibres and some fast twitch intermediate (type IIa) fibres (Essén, 1978; Gollnick, Armstrong, Saubert et al., 1973; Tsintzas & Williams, 1998; Vøllestad & Blom, 1985; Vøllestad et al., 1984), the recruitment of a greater proportion of fast twitch fibres throughout the W session training sets to compensate for the reduced availability of depleted slow twitch fibres may have enabled the subjects to complete the session. Support for a faster recovery of fast twitch fibres comes from a study by Vøllestad,
Blom and Gronnerod (1989) who investigated the rate of glycogen resynthesis in different muscle fibre types. Vøllestad et al (1989) reported that glycogen resynthesis was faster in type II than type I fibres in the first 90 minutes after cycling to exhaustion at 75% of VO₂ max.

An additional explanation as to why subjects were able to complete the W session of the CW sequence given the significant reduction in muscle glycogen content may be due to the contribution of additional muscle groups during the exercises (Coyle, 1995). During cycling, the majority of the force produced to turn the pedals is provided by the quadriceps, more specifically the vastus medialis and lateralis (Citterio & Agostoni, 1984; Ericson, Nisell, Arborelius, & Ekholm, 1985). The importance of this is that the C session may have reduced the level of glycogen depletion in the hamstrings compared to the quadriceps due to a lesser contribution to force production. Consequently, when completing the leg-press and leg-curl exercises, which involve the use of the hamstrings (Earle & Baechle, 2000), the reduced force producing capacity of the quadriceps due to a low glycogen content may have been overcome by the recruitment of additional motor units from the hamstrings and other muscles to enable the completion of the two exercises. The fact that the subjects struggled the most with the leg-extension exercise compared to the leg-press and leg-curl exercises tends to suggest that this may be a plausible explanation. It should be noted that whilst the subjects were able to complete the current strength training protocol, they might not have if a different protocol was utilised, such as one with a greater volume of repetitions like that used for hypertrophy. In light of this, muscle glycogen depletion cannot be ruled out as a contributing factor to the acute mechanism of residual fatigue during concurrent training regimes using different strength training protocols.

The present finding of reduced muscle glycogen levels at the 25-hour post the W session time point compared to pre-training day samples (WC = 73%, CW = 81%), suggests that the first phase of the biphasic restoration of muscle glycogen stores had occurred or was in progress (Friedman et al., 1991; Ivy, 1991; Pascoe & Gladden, 1996). The small but non significant difference between the two sequences at this time point, can be explained by the time elapsed between the finish of the C session and the taking of the muscle sample at the 25-hour post W training time point. There was a period of 19 hours for the WC sequence and 29 hours for the CW sequence, thereby providing an additional 10 hours for the CW sequence to replenish glycogen stores. However, in light of the fact the levels reached at this time point were still below pre-exercise levels, though not significantly, indicates that even with a high
carbohydrate diet as used by the subjects, was not enough to fully replenish muscle glycogen levels from the low values found following the C sessions. The incomplete restoration of muscle glycogen stores following training has also been found in other studies were subjects have completed endurance-orientated exercise (Costill, 1967; Costill et al., 1988) and may be due to the biphasic nature of muscle glycogen restoration, with a reduced rate of glycogen resynthesis as the level of restoration increases (Friedman et al., 1991; Ivy, 1991; Pascoe & Gladden, 1996).

The muscle glycogen content 25-hour following the W session still being below pre-exercise levels for both sequence days was an important finding due to the possible impact upon subsequent endurance-orientated training sessions on subsequent days, as pre-exercise muscle glycogen content has been shown to be an influencing factor in the time to fatigue and intensity during prolonged exercise (Gollnick et al., 1974; Grisdale et al., 1990; Ivy, 1991). Whilst earlier studies by Costill and colleagues on the effects of reduced muscle glycogen content on successive days of running (Costill, Bowers et al., 1971) and swimming (Costill et al., 1988) found that performance was not effected in the short term (i.e. over a period of days), the inability of the exercised muscle to return to a completely replenished state before the application of another stimulus may impede the overcompensation cycle that occurs following the application of the first stimulus. This impedance could stem from the inability of the body to repair damage to muscle ultrastructure, as reduced muscle glycogen levels have been found to impair the repair process, a process where the damage to muscle membranes involved in the exercise are thought to interfere with the ability of cells to uptake glucose (O'Reilly et al., 1987). The result of this altered recovery process and hence reduced level of overcompensation could be a reduction in the level of adaptation over time. Therefore, further research is required to determine if the continued reduction in muscle glycogen content over successive days of training interferes with subsequent training sessions and strength and/or endurance development.

6.4.4. Effect of the Sequence of Training on Hormonal Responses

It has been proposed that the adaptation to strength training is different when combined with endurance training due to the endurance component creating a more catabolic environment that impedes strength development (Bell et al., 2000; Leveritt et al., 1999). Similarly, this suggestion has been proposed for the impedance of endurance development, whereby the
strength training component may modify the endocrine environment normally associated with endurance training (Leveritt et al., 1999). Such differences in the hormonal environment are thought to influence the cellular changes related to protein synthesis and subsequent muscle fibre adaptations as well as substrate utilisation and endurance capabilities (Kraemer et al., 1995). The limited research completed to date on alterations in hormonal responses to concurrent training sessions (Bell et al., 2000; Craig et al., 1991; Kraemer et al., 1995) has hampered the ability to determine the role that changes in such hormones as testosterone and cortisol may have on strength and endurance development in concurrent training studies. Furthermore, the limitation of using sampling time points separated by periods of weeks (Bell et al., 2000; Craig et al., 1991; Kraemer et al., 1995) or large periods of time, similar to that used in Study 2 (the beginning and end of the day) of the current project, is that the magnitude and time course of the hormone responses immediately post the different training sessions is not known. An examination of this response pattern may provide a better understanding of how the hormonal responses to concurrent training sessions may cause the adaptations found after days and weeks of training. For this reason multiple sample collection points were included in this study to examine the time course of the testosterone and cortisol responses over a 24-hour period for the two training sequences and their corresponding control days.

Two control days (W and C only), were included in the study in order to provide an indication of the diurnal changes that occur following single sessions of strength and endurance training. The control C day resulted in an increase in plasma cortisol concentration immediately post-training before decreasing over the course of the day. This finding is consistent with the acute response to endurance orientated activity previously reported (Hackney et al., 1995; Kindermann et al., 1982; Ronsen et al., 2001; Viru et al., 1992). In the present study, the increase in cortisol following the 170 minutes of cycling was anticipated given the duration of the training session and the significant reduction in muscle glycogen content found following the training session, as outlined in the previous section. Both physiological stress and reduced muscle glycogen content associated with prolonged exercise are factors that have been shown to stimulate increased cortisol secretion (Kraemer, 2000b). The small though non-significant increase in testosterone immediately following the endurance cycling session has also been previously reported for endurance-orientated exercise (Hackney et al., 1995; Kindermann et al., 1982; Ronsen et al., 2001; Viru et al., 1992).
In contrast, the control W day of the present study resulted in a reduction in both testosterone and cortisol immediately post-training. This finding conflicts with previous findings (Gotshalk et al., 1997; Guezennec et al., 1986; Häkkinen et al., 1988; Kraemer, 1988; Kraemer et al., 1993; Kraemer et al., 1998; Weiss et al., 1983). The reduction in testosterone may be the result of a combination of factors including the protocol design of the W session and trained status of the subjects. Strength training protocols using low numbers of repetitions (high-intensity) and longer rest periods between sets, similar to the protocol used in this study, have been found to produce lower testosterone responses than protocols utilising a higher number of repetitions (moderate-intensity) and shorter rest periods (Gotshalk et al., 1997; Häkkinen & Pakarinen, 1993; Kraemer et al., 1990). Furthermore well-trained subjects have been known to produce reduced hormone responses than less trained subjects, a response attributed to a greater level of adaptation (Kraemer, 2000b). Consequently, given that the subjects in the present study were well-trained in conjunction with the high-intensity yet long rest periods between sets, may have limited the level of stress incurred and hence suppressed the testosterone response post-training.

In the current study, inclusion of the control days also enabled the hormonal response pattern following a single training session to be compared to the response pattern following two training sessions of different types. Comparison of the control C day and CW sequence day responses indicated no significant difference up to the commencement of the second training session. However, both testosterone and cortisol showed increased concentrations immediately after the W training session that remained elevated above control day values for one and two hours post-training, respectively. These findings differ to the responses found for the W training session during the control W day and WC sequence, where reductions in testosterone and cortisol concentrations were found post-training. Similarly, comparison of the control W day and WC sequence showed no significant difference between the trial days up to the commencement of the second training session. But as was the case with the CW sequence, the completion of the C session after the W session elicited a notable increase in both testosterone (38%) and cortisol concentrations (157%) which were greater than the control day responses (8 and 15%, respectively) when the changes are expressed as a percentage of pre-training values.

The present findings for both training sequences, of altered or increased testosterone and cortisol responses for the sequence days, suggests that the subjects experienced a higher stress
response during the second bout of exercise using the same muscle groups as those used earlier in the day. The fact that the subjects showed increased peak and mean HR responses during the W session following the C session as well as increased cardiorespiratory and RPE responses and reduced cycling efficiency during the C session following the W session, supports the notion of a greater physiological stress by the subjects during those training sessions completed second compared to when the same sessions were completed first.

To the current investigator’s knowledge this study is the first of its type to compare hormonal responses to two different training sessions completed on the same day using different training sequences, which makes direct comparison to other training studies using single or multiple daily training sessions of the same type of training difficult. However, given that both the W and C sessions used the same muscle groups in both training sessions, the findings were consistent with previous studies that have completed two training sessions in the one day using the same muscle groups of either endurance (Ronsen et al., 2001) or strength training (Häkkinen et al., 1988) separated by periods of three and four hours rest, respectively. Both these previous studies showed hormonal response patterns during the second training bout that were different to those observed following the first training bout and adds support to the suggestion that multiple daily training sessions of the same or different types alter the hormonal responses that have been shown to exist following single training sessions.

Ronsen and colleagues (Ronsen et al., 2001) attributed the hormonal changes to a number of factors, one of which was a reduction in muscle glycogen levels, a factor known to stimulate increased cortisol release in order to utilise energy from alternative sources via promotion of gluconeogenesis as well as mobilisation of triglycerides by lipolysis (Chattoraj & Watts, 1986; Galbo, 1992). The significant reduction in muscle glycogen levels in the present study strongly supports this suggestion, for as indicated previously, subjects commenced the W session following the C session with low levels of muscle glycogen compared to when the same session was completed first during the WC sequence. An element of residual fatigue should also be considered as a contributing factor to the increased hormonal response of both testosterone and cortisol during the second training sessions, for as already alluded to in the previous sections, a greater number of motor units are recruited during situations of muscle fatigue in order to maintain force production (Sale, 1987; Wilson, 1994a). In light of the fact that the amount of muscle mass involved in the exercise as well as the total amount of work performed during the training session determines the amount of exercise stress and hence
level of hormonal response (Kraemer, 2000b), then it would be fair to suggest that a greater level of stress would have occurred for those training sessions completed second in the training sequences.

The CW sequence in the present study resulted in a similar response pattern to that of the control C day for the T/C ratio, with the only exception being a shift in the peak during the day from 16:00 to 18:00 hour and a higher yet not significant anabolic orientated status as reflected by a higher ratio. However, the WC sequence did result in a non-significant suppression of the T/C ratio for the three hours following the second training session compared to the control W day. This suggests a reduced anabolic state for the WC sequence compared to the control W. In terms of the two control days, the pattern of change was very similar over the course of the recovery period with the only exception being a large rise in the ratio at the end of the day for the control W. However, the bulk of this change can be explained because of the large variability between subjects, as reflected by the large SD at that time point.

Due to the difference in duration of the strength and endurance training protocols in the present study, it is difficult to compare the hormonal responses of the two training sequences prior to the completion of the second training session at 15:00 hours. Comparison of the only two common morning time points at 07:00 and 12:00 hours revealed no significant difference between the two training sequences with both showing significant reductions in testosterone and cortisol across this time period. This result suggests that the differences in the response patterns after the first training session were temporal and of a short duration given that the C session finished only one-hour before this time point. However, comparison of the two training sequences after the completion of the second training session showed that the hormonal responses both in magnitude and duration were affected by the sequence and type of training completed. The testosterone response following the C session during the WC sequence resulted in a greater response (38%) immediately post training compared to the W session (14%) during the CW sequence. In addition, the WC sequence testosterone concentration remained elevated above that of the CW response over the duration of the recovery. A similar response pattern was also found for cortisol. Whilst the testosterone difference was not significant, the cortisol result was significant or approached significance (p< .066) for four of the five post-training time points. This finding suggests that completion
of a second training session in a day alters or has the potential to alter the hormonal response of the second training session and is effected by the sequence of training.

The T/C ratio comparison of the two training sequences in the present study revealed no significant difference between the two training protocols, which may be attributed in part to the large variability between subjects. However, the results did indicate that the CW sequence resulted in a reduced ratio early in the day following the C session before increasing notably over the hours following the W session compared to the WC sequence. This suggests that the type of training session completed second has the potential to alter the hormonal balance following that training session. Whilst the current findings support the notion put forward by Kraemer et al (1995) and others (Bell et al., 2000; Leveritt et al., 1999) that the endurance component of a concurrent training regime may create a more catabolic environment that impedes strength development, it appears that the order is the influencing component and not just the inclusion of endurance training in a strength training regime.

The current data showed that the testosterone and cortisol responses returned to near control day concentrations three hours following the second training session for both training sequences. This helps explain why no significant difference was found between the post-training hormonal responses in Study 2. The previous study (Study 2) used a different strength training protocol incorporating upper body exercises and thereby altering the intensity of the session, in addition to a shorter duration endurance training protocol. Factors shown to influence the magnitude of the hormonal response post-training via altering the amount of muscle mass involved in the exercise as well as the total amount of work performed during the training session (Gotshalk et al., 1997).

Because the intensity and duration of the individual training sessions as well as the combination of the training sessions in sequence did not significantly affect the resting testosterone or cortisol concentrations the morning following the trial days, suggests that the altered hormonal responses found during the trial days were temporal. However, it is worth pointing out that not all trial days responded the same. For example, when the post 07:00 hour time point for testosterone and cortisol are expressed as a percentage of the pre 07:00 hour time point, a different pattern of change is noted across the trial days. Testosterone for the control C and W days recovered to 93 and 92%, whilst the CW and WC sequence days recovered to 85 and 106%, respectively. In comparison, cortisol for the control C and W days...
recovered to 107 and 104%, whilst the CW and WC sequence days recovered to 91 and 90%, respectively. These findings suggest that the combining of strength and endurance training protocols as well as the sequence in which the sessions are completed may influence the recovery levels of selected hormones. Kraemer et al (1995) observed a greater cortisol response for a COMB group over a 12-week period compared to S and E groups only, which supports this suggestion. Therefore, the answer as to how acute changes in hormone responses interact with physiological adaptation of strength and aerobic components may be found by examining the accumulated effect of selected hormones like testosterone and cortisol over the course of days and weeks of repeated bouts of concurrent training exercise, a direction for future research.

6.4.5. Effect of the Sequence of Training on Muscle Gene Expression

The physiological and morphological adaptations that occur within skeletal muscle in response to long-term exposure to a training stimulus are due to changes in ‘gene expression, mediated by changes in the rate of transcription of specific genes and in the rate of synthesis of specific proteins’ (Williams & Neufer, 1996). These changes have been demonstrated in strength training programmes using humans where increases in the expression of MHC mRNA following weeks of moderate- and high-intensity strength training have been correlated with increases in strength (Willoughby & Pelsue, 1998). In terms of concurrent training programmes, changes in the expression of a number of genes associated with muscle fibre transformation and hypertrophy or metabolic processes may be altered when strength and endurance training are combined compared to when completed alone, thereby impeding the level of adaptation over time due to changes in the rate of protein synthesis. Whilst alterations in muscle fibre transformation and hypertrophy processes have been proposed as chronic mechanisms for the impedance of strength development in concurrent strength and endurance training studies, to date the sources of this impedance has received little attention. Therefore, a number of genes associated with muscle growth and metabolic functions within the muscle were examined in the present study to determine if they were affected by the sequence of concurrent training sessions. It should be pointed out that it is not the intention of this discussion to address the significance of the changes in each gene but to show that the sequence of completing strength and endurance training sessions on the same day alters the level of expression of the selected genes collectively following physical training.
6.4.5.1. Expression of Genes Associated with Muscle Growth

In addition to the sarcomeric protein genes (MHCI, MHCIIa and MHCIIx) which are involved in muscle hypertrophy processes following strength training (Periasamy et al., 1989), two MRFs (myogenin and MyoD) were included in the list of genes examined in this study, as they are known to be skeletal muscle specific transcription factors, thought to be involved in regulation of many of the myofibrillar genes (Baar et al., 1999; Florini et al., 1996; Schiaffino & Reggiani, 1996). The gene IGF1 & 2 was also included because of their proposed relationship with the control of local tissue repair, maintenance and remodelling (Goldspink, 1999). Whilst there was a large degree of variability between the subjects’ responses in the current study, the results of the two sarcomeric proteins (MHCI and MHCIIa) showed that they were not affected by the sequence of training, as no significant changes in mRNA content were found across the six time points for either the WC or CW sequence. The IGF1 & 2 gene also showed no significant difference between the time points for either training sequence.

In contrast, the MRFs, myogenin and MyoD were influenced by the sequence in which the strength and endurance training sessions were completed. Unfortunately the large variation in mRNA content between the subjects distorts the graphical results. However, in general, myogenin responded more to the CW sequence than the WC sequence with significant increases (~5-6 folds) in mRNA content at the one, three and 25 hours following the W session time points relative to the pre and post C time points. Whilst not as many MyoD time points showed significant changes as myogenin, it also responded more to the CW sequence with a significant change (~5 folds) for the 25-hour post W time point compared to the pre and post C and one- and three-hour post W time points.

The present study confirmed the findings of Psilander (2002) who showed that the sequence of completing concurrent training sessions does alter the expression of genes associated with muscle growth in humans. However, Psilander (2002) indicated that the sequence of WC caused a greater mRNA concentration for the genes myogenin, MHCI and MHCIIa across the 6-48 hours post-training time points compared to the CW sequence. The current findings do not support this finding, with no significant changes found for either of the training sequences for the sarcomeric protein genes and a greater response for the MRFs to the CW sequence. The only similar gene response between Psilander (2002) and the present study was for the
gene IGF1 & 2, which did not show any significant change for either sequence. The discrepancies between the current study and that of Psilander (2002) could be related to a number of factors including the type of strength training protocol used (strength versus hypertrophy), the duration of the strength and endurance training sessions (60 and 180 minutes versus 20 and 45 minutes), the proximity of the training sessions to each other (180 minutes versus 15 minutes) as well as the type of subject population used (well trained versus untrained) and the muscle sampling time points, respectively. The significance of these differences in study design being that they change the intensity of muscle contraction and possibly the contractile activity pattern imposed on the muscle, both of which have previously been shown to influence the level of expression of genes responsive to muscle contraction (Periasamy et al., 1989; Williams & Neufer, 1996).

Even though it is difficult to compare the training sequence responses for the sarcomeric protein genes to single strength training sessions, the present results were consistent with previous reports that MHC mRNA did not significantly change in the first 24 hours post-training (Chesley et al., 1992). However, it has been suggested that the stimulation of myofibrillar protein synthesis by strength training is mediated by more efficient translation of mRNA and not an increase in the level of gene expression (Welle, Bhatt, & Thornton, 1999). Therefore it is possible that a similar situation may apply to concurrent training sessions. Furthermore, it is also possible that the sacromeric protein genes may respond at a later time point than the 25 hours following the W session and possibly in response to the elevated MRFs. In view of the elevated level of myogenin and MyoD at the 25 hour time point for the CW sequence of the present study and the fact that these myogenic regulatory factors are thought to be important in the regulation of myofibrillar genes (Baar et al., 1999; Florini et al., 1996), it is conceivable that their response and the sacromeric protein gene response may be linked (Alway et al., 2001). To date this possible link remains unclear (Baar et al., 1999) and has not been examined following concurrent training in humans, thus requires further research.

6.4.5.2. Expression of Genes Associated with Metabolic Function

A number of studies using animals (rats and mice) have investigated the effects of exercise on the expression of genes associated with metabolic function and shown transient increases in HKII (O'Doherty et al., 1993), GLUT-4 (Neufer & Dohm, 1993; Ploug et al., 1990), UCP3
and LPL mRNA (Hamilton, Etienne, McClure, Pavéy, & Holloway, 1998). Similar transient increases in UCP3, PDK4, HKII (Pilegaard et al., 2002; Pilegaard et al., 2000) and LPL mRNA (Pilegaard et al., 2002; Pilegaard et al., 2000; Seip, Mair, Cole, & Semenkovich, 1997) have also been found in a limited number of investigations that have used humans. Whilst these studies were not concurrent training studies, like the present study, they do highlight that exercise can alter the level of gene expression post-exercise and may provide insight as to the findings of Study 3 which showed changes in mRNA content of some of the abovementioned genes. However, the degree of change varied considerably between genes and subjects and was also influenced by the sequence of training.

Even though a direct comparison cannot be made between the WC and CW training sequences because mRNA quantification was completed on a relative basis and not absolute, a number of patterns were found for the genes associated with metabolic functions in the present study. These include,

(i) The genes PDK4, HKII and LPL showed larger changes (mean fold) across the post-trial day time points compared to pre-trial day time point for the WC sequence than the CW sequence, respectively. Further, the magnitude of changes for the WC sequence were greatest for the time points three hours post the C session and 25 hours post W session.

(ii) The genes PDK4 and PGC1 showed large transient changes three hours post the C session for the WC sequence that reduced significantly by the 25 post W time point. A similar pattern was also found for the CW sequence but the changes were not as great and were not significant for PDK4.

(iii) HKII showed a significant change three hours post the C session (~10 fold) for the WC sequence, that remained significantly elevated at the 25 hour post W time point (~9 fold). The same was found for the CW sequence except that the change was notably less three hours post the C session (~6 fold) and had returned to near pre C levels by the 25-hour post W time point (~3 fold). A similar pattern was also found for the gene LPL but the changes were not significant.
(iv) The genes GYS and UCP3 showed minimal change (~1-2 fold) following either the W or C training sessions for either sequence.

Overall, the results of Study 3 suggest that the WC sequence produced the most notable changes following the training sessions, particularly with respect to the post C session time points. This suggests that the sequence in which concurrent training sessions are completed does affect the expression of certain genes associated with metabolic function. Whilst the present results indicated a difference between the training sequences, muscle glycogen content does not appear to be the determining factor for the responses found for the majority of the genes. If so, then there should be no difference between the trial days in terms of the pattern or level of expression due to no significant difference being found between the two sequence days either following individual like training sessions or collectively, as in the 25 post W time points for muscle glycogen content. This suggests that factors other than the level of muscle glycogen content may contribute to the expression of metabolic genes. This suggestion was put forward by Pilegaard et al (2002) following their examination of whether pre-exercise muscle glycogen content influenced transcriptional regulation of genes during recovery from endurance exercise. After completing two studies using low-intensity exercise over a period of 2-3 hours comparing reduced and normal muscle glycogen content in the exercising legs, Pilegaard et al (2002) found that even though muscle glycogen levels were similar following exercise in the reduced glycogen legs, there was a difference between the trials in terms of gene expression. Pilegaard et al (2002) proposed that a possible explanation for this finding was that low muscle glycogen levels could be related to signalling events triggered by elevated plasma free fatty acid (FFA) levels and/or utilisation. This concept was proposed after they found that plasma FFA and glycerol were significantly elevated in the reduced glycogen trials compared to the control trials along with a lower RER throughout exercise and recovery.

Even though, plasma FFA and glycerol were not measured in the current study, the shift in the substrate utilisation during the C session of the WC sequence from carbohydrate to lipid oxidation, as indicated by a downward shift in RER, supports this concept. In addition, the greater response by LPL and PDK4 following the C session of the WC sequence, which have been shown to facilitate the muscles’ ability to uptake FFA and lipids (Lithell, Orlander, Schele, Sjödin, & Karlsson, 1979; Preiss-Landl, Zimmermann, Hämmerle, & Zechner, 2002) and responded to elevated FFA levels (Wu, Peters, & Harris, 2001), also supports this
suggestion. These changes in substrate utilisation and LPL and PDK4 expression were not found to the same extent following the C sessions of the CW sequence where the RER remained stable. Therefore, it is possible that the expression of selected genes like LPL are linked to the type of substrate being utilised and its associated metabolic pathways. It is also possible that the difference in substrate utilisation for the two sequence days might not account for the response of some genes like GYS. Glycogen synthase is the catalyst for glycogen synthesis and as such responds to the level of muscle glycogen post-exercise (Ivy, 1991). Consequently, because similar muscle glycogen levels were found following the C sessions for both training sequences and that the completion of a W session did not seem to directly affect glycogen levels post-training, it is conceivable that the expression of this gene may be directly associated with muscle glycogen levels and therefore did not show a difference between the WC and CW training sequences.

Another possible explanation for the differences in expression of some of the genes (e.g. PDK4 and LPL) between the training sequences may be related to the altered hormone concentrations following the training sessions (Bamman et al., 2001). Even though no significant difference was found between the temporal increases in testosterone and cortisol following the training sessions completed second in the training sequence irrespective of the type of training, the WC sequence showed a trend towards higher hormonal concentrations than the CW sequence at the majority of the time points. The difference between the responses indicates that the level of physiological stress associated with the training sessions was not equal and probably reflects the differences in the intensity and duration of the training sessions. Therefore, it is conceivable that these hormone responses for the WC sequence may have influenced the level of expression of some of the metabolic genes.

6.4.5.3. Comparison of the Muscle Growth and Metabolic Genes

An interesting finding of the present study was that the genes associated with muscle growth responded more to the CW sequence whilst some of the genes associated with metabolic functions responded more to the WC sequence. Furthermore, the responses were generally greater following the second training session for both the muscle growth and metabolic genes. Whilst this suggests that the factors governing the response of the genes associated with muscle growth and metabolic functions may be different, it also suggests that the type of training session completed second in the training sequence may be important in determining
which genes are more affected. Subsequently, it is possible that the responses of the genes associated with muscle growth for the CW sequence were a reflection of the W session whilst the genes associated with metabolic functions were a reflection of the C session of the WC sequence. This suggestion is supported by the fact that across both the muscle growth and metabolic gene groups, a number of the genes showed greater fold changes at 25 hours post the W session compared to the pre-trial day time point for the CW and WC training sequences, respectively. Like the hormonal responses outlined in the previous section, it seems that the expression of a number of genes examined in this study are not only influenced by the completion of the concurrent training sessions but the order in which the sessions are completed.

The notable difference in expression at 25 hours following the W session between the training sequences for some of the genes is of particular importance because it has been suggested that the training induced transient increases in mRNA levels of genes and the cumulative effects of these changes during recovery determines the cellular adaptations (Pilegaard et al., 2000). The cumulative effects of transient increases in mRNA levels of metabolic genes was highlighted by Pilegaard et al (2000) in a study of repeated exercise bouts over consecutive days. It was found that after five days of 60-90 minutes of one-legged knee extensor exercise some genes (e.g. CPT1 and GYS) were elevated in the exercised leg compared to the non-exercised control. Therefore, one could speculate that subjects could possibly adapt differently to the training sequences, as used in the present study, if the response pattern of some of the genes examined (e.g. myogenin, MyoD, PDK4, PGC1, HKII and LPL) were the same on successive occasions. These differences in gene expression and the subsequent protein synthesis that follows may therefore be a contributing factor to differences in strength and/or endurance adaptations reported previously.

Whilst the present study suggested that the expression of genes associated with both muscle growth and metabolic functions are altered according to the sequence in which concurrent strength and endurance training sessions are performed, some important questions are still to be determined. First, what are the gene expression response patterns of single strength training compared to endurance training sessions? Second, what is the time course of the transient responses to current training sessions and is the response pattern altered by the proximity of the training sessions? Finally, is this response pattern the same on successive applications of the training sequence and is there an accumulative effect? These questions along with their
effect on protein synthesis need to be addressed in future studies before the influence that alterations in gene expression have on adaptation are fully known, following concurrent training sequences.

6.4.6. Summary and Conclusion

In summary,

- The C and W sessions of the WC and CW sequences both showed an increased physiological stress compared to when completed first in the training sequence, as indicated by elevated cardiorespiratory and HR responses during the exercise periods, respectively. Further, the physiological stress during the C session that followed the W session increased along with exercise time, possibly due to the accentuated effects of the prior W session on muscle fatigue and a change in cycling efficiency.

- A similar muscle glycogen content was found post the C session for both the WC and CW sequences. In contrast, a significantly lower muscle glycogen content was found pre W and 1 hr post W for the CW sequence compared to the WC sequence. The WC and CW sequences recovered to 73 and 81% of pre-training day levels 25 hours post the W session, respectively.

- Both sequence days showed temporal increases in testosterone and cortisol following the second training sessions compared to the control days. Even though no significant difference was found between the two training sequences, the CW sequence produced a higher T/C ratio and a delay in the peak response post the second training session compared to the C control day, whilst the WC sequence which produced a reduced T/C ratio compared to the W control day.

- The genes MyoD and myogenin responded more to the CW sequence whilst the genes PDK4, HKII and LPL responded more to the WC sequence. The remaining genes associated with muscle growth and metabolic function did not respond more to one training sequence than another.
It was concluded that,

- Irrespective of the type (W or C) or sequence (WC or CW) of training, the completion of a second training session three hours after a prior training session increased the level of physiological stress during that session.

- The sequence of training did not directly affect the level of muscle glycogen depletion post the training sessions. Further, that low muscle glycogen content prior to the commencement of the W session in the CW sequence did not prevent the completion of the session and that a period greater than 24 hours was required to fully restore muscle glycogen content after the bouts of prolonged cycling exercise accompanied by strength training either prior to or following.

- The completion of a second bout of training altered the testosterone and cortisol responses compared to a single training session, irrespective of whether it was strength or endurance orientated. Further, that the CW sequence produced a more anabolic state than the WC sequence in the hours following training.

- The level of expression of some skeletal muscle genes associated with growth and metabolic functions were altered by the sequence in which the concurrent training sessions was completed.
CHAPTER 7

General Discussion

7.1. Introduction

The three studies of this thesis investigated the effect of a number of training variables on muscle force generating capacity and cycling efficiency. Whilst some common factors were found to have contributed to the results including the subject training age and/or VO₂ max, residual muscle fatigue appears to have been the major factor, defined as exercise-induced reduction in the ability of a muscle to generate or maintain force or power (Gandevia, 2001; Maclaren et al., 1989). Fatigue is a multi-dimensional and complex phenomenon that can originate from a large array of sources ranging from metabolic factors such as the accumulation of metabolites and substrate depletion to non-metabolic factors including impairment of neuromuscular pathways and muscle damage (Maclaren et al., 1989; Pyne, 1994). Fatigue can also be caused by environmental conditions such as heat, cold and altitude (Pyne, 1994). It is not the intention of this General Discussion to discuss all the factors contributing to fatigue. However, those factors that contributed to the results or considered by the literature to contribute to the interference of concurrent training sessions will be discussed. To effectively address these issues, this Chapter has been divided into three areas: (1) factors contributing to the reduction in force generating capacity; (2) factors contributing to the increased physiological cost during the concurrent training sessions including factors affecting cycling efficiency; and (3) residual fatigue as a possible source of long-term interference in concurrent training adaptation.

7.2. Factors Contributing to the Reduction in Force Generating Capacity

An examination of maximal voluntary contractions were included in Study 1 and 2 of the present thesis to assess the recovery of force generating capacity following each of the training protocols and sequences due to the different intensity levels and types of stress loading between the various training protocols (concentric/eccentric and concentric only), that
have previously been shown to influence the rate of recovery post-exercise (Folland et al., 2001; Newham, Jones et al., 1983). However, unlike previous studies that have assessed muscle force generating capacity immediately following training (Häkkinen, 1992; Häkkinen & Pakarinen, 1993; Häkkinen et al., 1988), testing in the present studies was carried out three hours post-training to determine the recovery capacity of the muscle prior to the completion of another exercise session (Study 1 - the cycling efficiency test, Study 2 – the second training session). A scenario that often occurs with athletes who undertake endurance sports and utilise strength training to supplement their training by completing an endurance training session on the same day after a period of rest.

The results of Study 1 suggest that force generating capacity had not recovered to pre-exercise levels three hours following the four training protocols, particularly for the S, SUL and H protocols. The results of Study 2 also suggest a similar finding between and following the two training sequences, with the WC sequence showing the greater reduction in force generating capacity compared to the CW sequence. The reduction in muscle force generating capacity post-exercise has been attributed to many factors including accumulation of metabolic by-products, depletion of fuel sources, disturbance in the internal environment, muscle damage and impaired neural control of muscle contraction (Allen et al., 1992; Grisdale et al., 1990; Maclaren et al., 1989; Pyne, 1994; Sahlin, 1992).

The accumulation of lactic acid and/or a reduction in pH were unlikely factors in the reduction in force generating capacity in Study 1 and 2 because of the time that had passed between the finish of the training protocols and MVC trials, which would have allowed sufficient time for lactic acid to be dispersed (Baker et al., 1993; Bangsbo et al., 1994) and for pH to have returned to pre-exercise levels (Juel, Bangsbo, Graham & Saltin, 1990). The low resting BL concentrations prior to the efficiency test in Study 1 and the second training sessions in Study 2, confirm this suggestion. This is not to say that lactic acid accumulation and/or a reduction in pH would not influence concurrent training sessions where strength training sessions, such as the S (4.85 ± 1.79 mmol.L⁻¹) and SUL (7.29 ± 1.17 mmol.L⁻¹) protocols with moderate to high post-training BL concentrations, are completed immediately prior to an endurance training session as was the case with the studies by Collins and Snow (1993), Dolezal and Potteiger (1998); Gravelle and Blessing (2000) and Nelson et al (1990).
Even though muscle glycogen content was not assessed in Study 1 or 2, it is unlikely that a reduction in muscle glycogen content was responsible for the reduced force generating capacity following the strength training sessions because of the short duration (30-60 minutes) of the training sessions, the use of only one leg-exercise for strength training and the time period between the finish of the training protocols and MVC trials. The results of Study 3 confirmed this, in that the strength training protocol consisted of three high-intensity leg-exercises and did not cause any significant change in muscle glycogen content one-hour post-training. Furthermore, previous studies that have reported significant reductions in muscle glycogen content post-strength training exercise have utilised a greater volume of training consisting of a higher number of repetitions (Pascoe et al., 1993) and/or leg-exercises (Tesch et al., 1986). Similarly, in view of the moderate intensity and short duration of the C session in Study 2, it is also unlikely that muscle glycogen depletion was the cause of the reduction in force generating capacity for the WC sequence. Studies that have examined muscle glycogen content over various durations and intensities of exercise have shown that there are less notable changes during one-hour of cycling at moderate intensities (Gollnick, Armstrong, Saubert et al., 1973; Vøiestad & Blom, 1985) compared to higher intensities (Hultman & Greenhaff, 1992; Newsholme et al., 1992; Stainbsy, Gladden, Barclay, & Wilson, 1980; Vøiestad & Blom, 1985) and periods greater than two hours, which have been found to produce substantial reductions in muscle glycogen content (Gollnick, Armstrong, Saubert et al., 1973; Hultman & Greenhaff, 1992; Newsholme et al., 1992). The large reduction in muscle glycogen for the three-hour cycle sessions in Study 3 also supports this suggestion. Furthermore, reductions in force generating capacity have been found to persist for days following exercise even though muscle glycogen levels had returned to the pre-exercise levels (Sherman et al., 1984).

This suggests that the inability to generate maximum force might be due to other sources including damage to structural elements of the muscle or to other non-metabolic factors (Sahlin & Seger, 1995). The more likely reason for the reduction in force generating capacity following the training protocols in Study 1 and 2 was some form of muscle damage or neuromuscular fatigue. Previous research has identified that muscular fatigue may be caused by either central or peripheral factors (Maclaren et al., 1989; Sahlin, 1992). Central factors are those processes that control the discharge rate of motor neurons or more simply the failure to activate the muscle voluntarily (Gandevia, 2001), whilst peripheral factors refer to those processes distal to the neuromuscular junction (Gandevia, 1992). Consequently, in order to
determine if any reduction in maximal force generating capacity following the training protocols and sequences was due to central or peripheral fatigue, a twitch interpolation technique was conducted in conjunction with the MVC trials in Study 1 and 2. The advantage of this technique is that it allows “the central drive to the muscle to be determined, in addition to the peripheral force generating capacity of the muscle” (Allen, McKenzie, & Gandevia, 1998).

The reductions in MVC peak and mean torque along with the parallel reductions in the superimposed and control twitches for both Study 1 and 2 suggests that the reduction in force generating capacity following the training sessions were due to peripheral fatigue. Whilst the exact source of the peripheral fatigue in the studies is unknown it could have been due to damage to the muscle contractile structures (Z discs and myofilaments) inhibiting muscle contraction force (Armstrong et al., 1983; Behm et al., 2001; Fridén, Sjöström et al., 1983; Stauber, 1989). The greater changes in CK found for the S and SUL training protocols in Study 1, which also showed the greatest reduction in peak and mean torque compared to the notably lesser CK responses for the SE and H protocols, supports this line of reasoning. However, if the reductions in MVC torque are related to the level of muscle damage, as reflected by an increase in CK, why then was there not a greater increase in CK for the H protocol given the similar reduction in MVC torque as found for the S and SUL protocols? Similarly, why wasn’t there a greater increase in CK for the WC sequence in Study 2 given that a greater reduction in MVC torque, though not significant, was found over the course of the trial day? These findings suggest mechanisms other than direct damage to muscle contractile structures may have also contributed to the result, such as neuromuscular disruptions.

One of the neuromuscular mechanisms attributed to the loss of force generating capacity is the failure of excitation-contraction coupling as a result of disruption of the surface membrane and t-tubule system, failure of the coupling mechanism between the action potential and the release of calcium or the failure of calcium regulation at the level of the contractile elements (Allen et al., 1992; Enoka & Stuart, 1992; Fitts & Balog, 1996; Jones, 1996; Stauber, 1989). Whilst the failure of excitation-contraction coupling is generally associated with loss of force generating capacity following eccentric exercise (Morgan & Allen, 1999), similar disruptions have also been found in animal research (Giddings et al., 1985) following high-muscle tension during concentric exercise, probably due to the increased tension per unit of muscle.
(Newham, McPhail et al., 1983). This may be the reason why the reduction in peak and mean torque for the H protocol was similar to that of the S and SUL protocols. The development of muscle tension and its association with disruption to neuromuscular function may also explain why there was a difference between the significant reduction in mean torque found following the H training protocol and the minimal changes found for the C session in Study 2, even though a greater volume of work was completed for the 60-minute C session. Similarly, the difference in the level of MVC torque following the W and C sessions for the WC and CW training sequences respectively, may also be explained by the difference in the intensity of the exercise and subsequent greater level of muscle tension for the W exercise compared to the cycling exercise (Gibala et al., 1995). The higher post-training BL concentration for the W session (5.51 ± 1.42 mmol.L⁻¹) compared to C session (1.85 ± 0.22 mmol.L⁻¹) supports the suggestion of a greater level of tension due to a greater contribution from fast twitch fibres.

The discussion so far has addressed the issue of peripheral fatigue. However, previous research has shown that a central fatigue component may also contribute to reductions in force generating capacity post-exercise (Davis & Fitts, 1998). This is typically indicated using the twitch interpolation technique by an increase in torque achieved in the superimposed twitch response during the MVC (Allen et al., 1995b; Green, 1995). The present results of the voluntary MA calculations showed very little change for any of the protocols or sequences, this finding suggests that the contribution of central fatigue to the reduction in MVC torque was minimal. The minimal contribution of a central fatigue component to the results may be due to the duration and/or intensity of the training protocols. Even though the S and SUL protocols were classified as high-intensity, the leg- exercise component was low in volume. In contrast, the C sessions for Study 2 were longer in duration but the intensity only moderate. These differences may explain why no central fatigue component was found in the present studies, because those previous studies that have identified central fatigue as contributing to the reduction in force generating capacity have used high-intensity cycling (Bentley, 1998) or strength training protocols with a greater training volume (Häkkinen, 1992, 1993; Linnamo et al., 1998). Such training regimes may stress the central nervous system more than the lower intensity and/or volume training protocols as used in the present studies. In light of this, a central fatigue component may have contributed to the results of Study 3 in view of the intensity and number of leg-exercises in the W session along with the prolonged duration of the C session.
In conjunction with the minimal contribution of central fatigue to the reductions in MVC torque, the contribution of a reduction in voluntary MA in the present studies also seems to have been minimal. However, the current results may also be attributed to the method of calculation of the level of voluntary MA. The equation used to calculate the level of voluntary MA in Study 1 and 2 is based on dividing the superimposed twitch by the control twitch, as outlined in the General Methodology and Materials Chapter (Section 3.3.3). Because the superimposed twitch and the control twitch were both decreasing in conjunction with a decrease in MVC torque, means that the level of voluntary MA would be reducing proportionally. This explains why no significant difference was found between the training protocols for the level of voluntary MA despite a significant reduction in MVC torque being found for the S, SUL and H protocols in Study 1. Moreover, it may also explain the findings of Study 2 where a similar result was found.

Even though no significant reduction in voluntary MA was found for Study 1 or 2, the level of voluntary MA for the various training protocols and sequences were consistent with those previously reported for the quadriceps (Babault et al., 2001; Roos, Rice, Connelly, & Vandervoort, 1999; Shield, 2003). The results also confirm the suggestions that full activation of a muscle does not happen regularly (Belanger & McComas, 1981; Gandevia, 2001) and that there is a lot of variability between subjects with some subjects being able to achieve greater levels of activation than others (Babault et al., 2001; Roos et al., 1999; Shield, 2003).

The differences in the reduction in force generating capacity between the various training protocols and sequences are consistent with the concept of task dependency that suggests the mechanisms underlying fatigue vary from one task to another (Enoka & Stuart, 1992), as demonstrated by the differences in fatigue following various activities (Behm, Anderson, & Curnew, 2002; Linnamo et al., 1998). It should also be remembered that fatigue is rarely due to just one factor and as such a combination of the previously discussed factors may have contributed to the results in some way (Enoka, 2002; Enoka & Stuart, 1992). For example, the reduction in MVC following the SUL protocol in Study 1 can be attributed to both damage to contractile elements and disruption to failure of excitation-contraction coupling due to the eccentric contraction and high-intensity load (Bigland-Ritchie & Woods, 1984; Morgan & Allen, 1999). A combination of factors may also be the reason why the C session of the WC sequence produced a greater reduction in mean torque compared to the CW sequence, which showed minimal change.
7.3. Factors Contributing to the Increased Physiological Cost during the Concurrent Training Sessions

It is well documented (Burleson et al., 1989; Elliot et al., 1992; Melby et al., 1992; Murphy & Schwarzkopf, 1992; Thornton & Potteiger, 2002) that metabolism can remain elevated following a bout of strength training due to an exercise-initiated disturbance of the body’s pre-exercise state (Brooks et al., 2000). Further, the magnitude and duration of this disturbance is affected by the intensity and duration of exercise (Bahr, 1992; Børsheim et al., 1998). Whilst these studies have examined the time course taken for metabolism and EPOC to return to base-line levels following a bout of strength training, no studies have examined the influence that these exercise-initiated disturbances may have on sub-maximal cycling performance. Research of multiple endurance cycling training sessions in one day separated by a period of three hours rest (Ronsen et al., 2001) has shown that there is an increased physiological cost during the second training session compared to the initial training session. A similar finding has also been found with respect to cycling exercise completed in the days following a bout of eccentric exercise compared to concentric exercise (Gleeson et al., 1995). In light of the above findings, in the current series of studies a bout of sub-maximal cycling was completed three hours following the strength training sessions in Study 1 as well as the second training session in Study 2 to determine if the recovery dynamics from the various types, modes, durations and sequences of training would affect subsequent sub-maximal cycling performance. Similarly, respiratory responses were measured during the C sessions in Study 2 and 3 in order to determine if cycling efficiency was affected by the prior strength training sessions.

The findings of all three studies suggested that there was an increased physiological cost to varying degrees, depending on the type, duration and mode of the prior strength training protocol, during the rest and exercise periods of the efficiency tests or C sessions. Furthermore, because a number of the physiological variables (RR, $V_E$, $VO_2$ and BL) responded more to the higher workloads of the efficiency tests in Study 1 and 2 and the duration of the C session in Study 3 following the high-intensity strength training sessions, suggested that different factors may be contributing to the resting and exercising responses. Overall, the elevated physiological response during both the rest and exercise periods for the efficiency tests and C sessions in the respective studies indicated that a greater period of time
was required to bring the physiological processes back to equilibrium than the three hours provided between training sessions in the current studies and support the findings of previous research (Thornton & Potteiger, 2002).

7.3.1. Factors Affecting Resting Metabolism

Even though the exact mechanisms for the prolonged elevation of a number of the physiological variables (HR, VO$_2$, V$_E$ and RR) in the present series of studies are not known, it may be due to one or a combination of factors including the repair of damaged tissue (Børsheim et al., 1998; Schuenke et al., 2002), residual effects of elevated hormone levels (Bahr, 1992; Bangsbo & Hellsten, 1998; Mahlum et al., 1986) and/or a changes in substrate utilisation (Bahr et al., 1990; Wolfe et al., 1990).

Eccentric loading has been shown to produce higher levels of muscle damage than concentric loading (Armstrong et al., 1983; Clarkson et al., 1985; Hortobágyi & Denahan, 1989; Jamurtas et al., 2000; Newham, McPhail et al., 1983). Because the repair of muscle damage requires an increase in protein breakdown and synthesis, an increased amount of energy is also required (Dolezal et al., 2000; Melby et al., 1992). An increased energy cost requires an increase in oxygen consumption to match oxygen demand for the repair process (Børsheim et al., 1998; Schuenke et al., 2002). This factor may help explain the small difference in pre-exercise metabolism between the high- and low-intensity training sessions as well as the H protocol for the efficiency tests in Study 1, given the differences in CK activity 24 hours post-exercise.

Though not extensively previously researched (Mahlum et al., 1986), it has been proposed that the residual effects of elevated hormone levels such as cortisol, insulin and catecholamines, may alter metabolic processes during the recovery period post-exercise and thus contribute to an increase in energy expenditure (Bahr, 1992). It is not possible to tell from the protocols used in the current series of studies if elevated hormone levels contributed to the results due to only having measured two hormones (testosterone and cortisol), the absence of a larger number of sampling points (Study 2) or a control day without exercise (Study 3). However, the hormonal results in Study 3 support this suggestion as a possible contributing factor since cortisol, a hormone that influences substrate selection post-exercise, was found elevated following the second training session for both sequence days in Study 3.
compared to the single training session days. In association with possible changes in hormone levels post-exercise, a change in substrate utilisation may also be a factor as indicated by the reduction in RER towards more lipid utilisation for those C sessions that followed the W sessions in Study 3. An increase in fat oxidation suggesting an increase in oxygen consumption since the energy equivalent of oxygen is lower for fat compared to carbohydrates (Bahr, 1992). Similar changes were found for the WC sequence in Study 2 as well as the efficiency tests following the high-intensity strength training protocols in Study 1 though less noticeable. In summary, these findings are consistent with previous reports that have found changes in hormones (Fahrner & Hackney, 1998; Gotshalk et al., 1997; Hartley et al., 1972; Kindermann et al., 1982) as well as reductions in RER post-exercise (Bahr et al., 1987; Bahr & Sejersted, 1991) to be related to intensity and duration of exercise.

It is also possible that an elevated body temperature may have been a contributing factor to an elevated resting metabolism in the current series of studies, as suggested by the significantly higher pre-training TT measurement for the WC sequence in Study 2. An elevated body temperature decreases phosphorylation coupling efficiency and requires a greater amount of oxygen for a given amount of ATP to be synthesised (Brooks et al., 1971). Even though there is evidence to show that there is a relationship between EPOC and an elevated core temperature post-endurance exercise (Neary, Docherty, & Wenger, 1993), it has been found to be short in duration (< 1 hour), which is why the thermogenic effects of elevated body temperature are generally associated with the rapid component of EPOC (Brehm, 1988; Brooks et al., 2000; Brooks et al., 1971; Gaesser & Brooks, 1984; McArdle et al., 1996). However, this is not to say that a greater increase in the duration of this relationship following high-intensity strength training may occur. The results of Study 1 support this suggestion, because a slightly higher resting TT was found for the SUL protocol compared to the other training protocols three hours post-training.

7.3.2. Factors Affecting Cycling Efficiency

Energy expenditure and efficiency during cycling exercise have been extensively investigated and found to be influenced by a number of external factors including bicycle positioning and equipment (Hagberg & McCole, 1990; McCole, Claney, Conte, Anderson, & Hagberg, 1990), cadence (Hagberg et al., 1981; Marsh, 1995), workload (Coast & Welch, 1985; Seabury et al., 1977) and environmental conditions (Galloway & Maughan, 1997). Cycling efficiency has
also been found to be influenced by a number of internal factors including muscle fatigue (Passfield & Doust, 2000) and the type and percentage of muscle fibres recruited for exercise (Coyle, Sidossis, Horowitz, & Beltz, 1992; Horowitz, Sidossis, & Coyle, 1994; Suzuki, 1979) as well as the amount of energy expanded during resting metabolism and for unmeasured work during exercise (Sawka, 1986). In the current series of studies, the factors that appear to have contributed to the changes in cycling efficiency were the internal factors because the abovementioned external factors (bicycling position and equipment, cadence, workload and environment conditions) were controlled during the efficiency tests and C sessions. Two other factors that may have also influenced cycling efficiency during the efficiency tests and C sessions were alterations in motor unit recruitment patterns due to the prior training sessions and the subject training age and/or VO₂ max. These later factors are discussed separately.

Generally, the increased physiological cost of cycling exercise is most evident by a reduction in GE as it is a representation of whole body efficiency and as such an increase in resting metabolism, energy expenditure for measured work or unmeasured work without a corresponding increase in work output will result in a reduction in efficiency (Sawka, 1986; Sidossis et al., 1992). The notable reduction in GE for the C session of the WC sequence compared to the CW sequence in Study 2 and 3 confirmed the presence of an increased physiological cost of the exercise following the prior bout of high-intensity strength training. The efficiency test results of Study 1 also showed that there was an increased physiological cost of cycling for the four strength training protocols as indicated by the elevated responses for a number of the physiological variables. However, unlike the C sessions of Study 2 and 3, the changes in GE and NE efficiency were small and insignificant. This suggests that the disturbance to resting and/or exercise metabolism were of insufficient levels to significantly effect GE or NE three hours post-training at low-moderate intensity workloads.

Unfortunately, because of the method used to calculate GE in the present studies, it is not possible to determine whether a change in GE during an exercise period are due to a changes in resting metabolism and/or energy expenditure during exercise (Gaesser & Brooks, 1975). However, in view of the elevated respiratory variables (RR, V̇E and VO₂) during both the rest and exercise periods of the of the efficiency tests in Study 1 and 2 and for the C session of the WC sequence in Study 2 and 3, it would appear that the deterioration in efficiency was due to an increase in both resting and exercising energy expenditure. The NE results, if viewed in conjunction with the GE results, also suggest that the percentage of contribution from these
two sources varied between the studies. It should also be noted that the increase in energy expenditure could have also been due to additional energy expenditure for unmeasured work from such sources as torso stabilisation or isometric contractions (Sawka, 1986), as a consequence of the high-intensity leg and/or upper body strength exercises fatiguing other muscle groups used in torso stabilisation during the cycling exercise.

During the C session of the WC sequence in Study 3, the NE results suggested that the majority of the increased energy cost was associated with the completion of the cycling exercise and not resting metabolism. The reason for this statement is that the equation used to calculate NE is based on the subtraction of the energy required for the resting component of the exercise from the total energy expended during the exercise (Gaesser & Brooks, 1975; Sawka, 1986). Even though this method makes the assumption that resting metabolism remains constant over the course of the exercise period, normally any increase in the proportion of resting metabolism results in an increase in efficiency as a greater proportion of energy expenditure is deducted (Stainbsy et al., 1980). Because the NE results for the WC sequence averaged 1.64% below the CW sequence means that the amount of energy expenditure deducted for the resting component from the total energy expended was notably smaller than the proportion used during the exercise. This suggests that even though there was a large disturbance of resting metabolism, as indicated by the elevated resting values for a number of the physiological variables prior to the C session, there was an even greater disturbance to exercising metabolism. The GE results for the WC sequence in Study 2 were also below that of the CW sequence, similar to that found for Study 3 however, the NE results were slightly higher or similar at the various time periods. This suggests less of a contribution to the increased physiological cost of exercise from an increase in exercise metabolism than that found in Study 3. The reasons for this difference between Study 2 and 3 are outlined later in this discussion.

The factors contributing to an elevated resting state, as outlined in the prior section, may have also contributed to the exercise period of the efficiency tests and C sessions in the respective studies along with the W sessions that followed the C sessions in Study 2 and 3. However, the contribution of each factor to the increased physiological cost of exercise and changes in efficiency probably varied according to the level of stress of the prior workload (intensity or duration) similar to that reported for EPOC studies following various strength (Schuenke et al., 2002; Thornton & Potteiger, 2002) and endurance (Bahr et al., 1987; Gore & Withers,
1990) training protocols. Apart from an elevated resting metabolism, the other factor that appears to have contributed to the physiological cost of the cycling as well as the W training sessions post the C sessions, was the level of muscle fatigue from the prior training sessions. The sources of this fatigue being the same as those that contributed to the reduction in force generating capacity following the various training protocols, as outlined earlier.

Muscle fatigue is an important consideration in determining the physiological cost of exercise because a greater exercising muscle mass is recruited to maintain power output in accordance with the level of muscle fatigue (Coyle, 1995; Sale, 1987; Wilson, 1994a). Along with an increase in the amount of muscle mass, there is an increase in energy expenditure due to changes in the type and proportion of muscle fibres recruited for exercise, with a greater contribution to power production by more powerful fast twitch fibres (Sale, 1987; Wilson, 1994a), as outlined in the respective discussions for Study 1 and 2. Apart from causing an increase in muscle temperature and lactate accumulation (McArdle et al., 1996; Seabury et al., 1977), there is evidence to indicate that muscle efficiency is reduced as more fast twitch fibres are recruited, which have lower contractile-coupling efficiency than slow twitch fibres (Kang et al., 1997; Whipp & Wasserman, 1969). Consequently, it is possible that the increased physiological cost and subsequent reduction in cycling efficiency in the current series of studies was due to a reduction in muscle efficiency as a result of muscle fatigue. Even though it is not possible to determine whether a reduction in muscle efficiency contributed to the current findings from the GE and NE calculations, the increased responses of some of the other variables measured support this concept as a possible explanation.

The elevated BL concentration throughout the efficiency tests following the various strength training protocols in Study 1, the C sessions of the WC sequence in Study 2 as well as the efficiency tests following the two sequence days in Study 2, which corresponded with a shift in RER towards anaerobic metabolism (Gaesser & Brooks, 1975) as well as the reductions in MVC torque prior to the efficiency tests or C sessions, indicated a contribution from a greater percentage of fast twitch fibres. In addition, the strength training protocols that had the greatest effect on muscle fatigue, as reflected by the greater reductions in MVC torque post the training sessions, also showed the greatest physiological cost during the efficiency test except for the H protocol. This finding further supports the contribution of fatigue to the changes in cycling efficiency. The reason why the H protocol did not increase the physiological cost of the cycling in the efficiency test similar to the S and SUL protocols in
Study 1 is discussed below under the heading ‘The Alteration of Motor Unit Recruitment Patterns following Prior Training Sessions’. The same relationship was found for Study 2 where the sequence with the greatest reduction in force generating capacity also showed the most effect during the efficiency test compared to the control test.

The level of muscle fatigue may also explain the differences between the physiological responses of the C session for the WC sequence for Study 3 and that found for Study 2 as well as the large differences between the two training sequences in Study 3. These differences may be attributed to two factors. First, the type of strength training protocol utilised and second, the duration of the C session. Even though the duration of the W sessions was equal between Study 2 and Study 3 (60 minutes), the type of exercise performed was different. Both studies involved the completion of the leg-press. However, Study 2 involved two upper body exercises compared to Study 3, which involved two other leg-exercises. The other leg-exercises may have increased the amount of muscle tension generated in the leg-muscles over the 60-minute session, as the leg-press is a multi-joint exercise using both the quadriceps and hamstrings whilst the leg-extension and -curl exercises are single-joint exercises involving the same muscle groups (Baechle et al., 2000). Consequently, it is conceivable that the three leg-exercises used in Study 3 may have increased in the level of fatigue in the major muscle groups related to cycling - the quadriceps and hamstrings (Citterio & Agostoni, 1984; Ericson et al., 1985).

If the level of muscle fatigue was increased due to the additional leg-exercises, then the amount of muscle fibre recruitment and possibly the pattern of recruitment during the subsequent C session may have also been affected (Takaishi et al., 1994). Furthermore, that the increased physical stress of the W session, as a result of the increased muscle mass usage may have caused a greater disturbance of resting metabolism then that found in Study 2. This has been demonstrated previously when comparing strength training protocols of varying intensities requiring the use of different amounts of muscle mass (Schuenke et al., 2002; Thornton & Potteiger, 2002). In view of the above changes to the W training session, the physiological responses may have increased for the C session as time progressed due to a greater level of fatigue in the exercising muscles (Galloway & Maughan, 1997). The notable increase in the respiratory responses after 90 minutes of exercise as well as the progressive reduction in GE and NE over the duration of the C session for the WC sequence supports this suggestion.
The duration of the C session in Study 3 may have also contributed to the greater disturbance to exercising metabolism than that found for Study 2, because unlike Study 2 where there was an increase in the RER ratio for the WC sequence over the duration of the C session, Study 3 showed a reduction. The difference may be attributed to the level and/or rate of muscle glycogen depletion in response to the greater level of active muscle mass along with a possible change in the cortisol response as indicated by the large increase in cortisol post-exercise in Study 3. Cortisol responding to alter the substrate being utilised due to the low muscle glycogen content and/or rate of muscle glycogen depletion (Chattoraj & Watts, 1986; Galbo, 1992; Kraemer, 2000a). Even though the calculation of gross and net efficiency takes into account changes in substrate utilisation (Passfield & Doust, 2000), the large increases in the respiratory responses for the WC sequence above that of the CW sequence means that the total increase in energy expenditure for the same power output was considerably larger causing a reduction in GE and NE.

The significant reduction in muscle glycogen content for the C session of the CW sequence in Study 3 may also explain the difference found between Study 2 and 3 with respect to the BL concentration post-exercise. It was anticipated that an increased BL concentration would be found following the W session for the CW sequence in Study 3 because of residual muscle fatigue following the completion of the C session requiring a greater contribution of glycolytic fibres throughout the strength training sets, as found for Study 2. At first glance, it would appear that the results do not support this suggestion because the sequence of training did not appear to alter the BL concentration following the W session, as no significant difference was found between the WC and CW training sequences. However, some caution must be used when interpreting this finding as previous research on BL accumulation during short duration, intense exercise undertaken after the completion of prolonged exercise, has shown that BL concentrations can be significantly reduced (Jacobs, 1981; Le Gallais et al., 1999; Passfield & Doust, 2000; Saltin & Hermansen, 1967).

Passfield and Doust (2000) investigated the effects of moderate-intensity endurance exercise (60 minutes of cycling at 60% of VO₂ max) on 30-second sprint power output performed after exercise. They found the post-sprint BL concentration was significantly reduced by approximately 2 mmol.L⁻¹ following endurance exercise compared to when sprint performance was completed before. The reduction in post-exercise BL accumulation was
thought to be due to depleted glycogen stores resulting in increased BL utilisation (Bangsbo et al., 1992), as opposed to a reduction in BL production (Jacobs, 1981). The increased utilisation of BL as a fuel substrate seems the more plausible scenario to explain the current findings in Study 3. This is suggested due to the finding of a significant glycogen depletion of approximately 71% of pre-exercise levels following the C session. Furthermore, a reduction in BL production implies a reduced contribution from fast twitch glycolytic fibres, which seems unlikely in view of the subjects prolonged cycling exercise three hours prior and the evidence to indicate that there is a significant reduction in force generating capacity following prolonged cycling exercise (Lepers et al., 2000; Sahlin & Seger, 1995).

A synthesis of the findings of the three studies suggest that the factors that appeared to contribute most to the increased physiological cost and subsequent changes in efficiency during the cycling components of each study were an elevated resting metabolism and muscle fatigue from the prior strength training sessions, requiring the recruitment of a greater amount of exercising muscle mass in the subsequent exercise bout. The results of Study 3 also suggest that a higher intensity strength training session combined with a longer duration C session can substantially alter the physiological cost of sub-maximal cycling as well as reduce GE and NE. This appears due to the interaction of a number of fatigue related factors including a reduction in muscle glycogen levels, increased hormonal responses and a shift in substrate utilisation. The differences in the pattern of change with respect to GE and NE between Study 2 and 3 highlighted that the duration and intensity of the concurrent training sessions may be a major factor in determining the extent to which the residual effects of the prior training sessions have on subsequent training sessions.

7.3.2.1. The Alteration of Motor Unit Recruitment Patterns following Prior Training Sessions

The increased physiological cost and changes in cycling efficiency for the efficiency tests in Study 1 and 2 and the C sessions following the W sessions during the CW sequences in Study 2 and 3 could have also been affected by alterations in motor unit recruitment patterns. It is possible that the combination of prior strength training then cycling exercise altered the motor unit recruitment patterns normally associated with cycling, causing more inefficient motor units to be recruited to perform the same amount of work (Bangsbo, 1996; Ebbeling & Clarkson, 1989). This effect may be similar to the changes observed in running mechanics.
following cycling in triathlon (Gottschall & Palmer, 2000; Guezennec et al., 1996) or the change in motor unit recruitment patterns observed for different cycling cadences, where the proportion of muscle contribution to force production varies with increases in cadence (Takaishi et al., 1994).

The findings of the present study cannot confirm the presence of altered motor unit recruitment patterns for the training sequences. However, the current findings do suggest the possibility that motor unit recruitment patterns may have been altered, particularly for where conventional strength training is completed prior to endurance training. Support for this concept can be found in the reduced influence that the H protocol had on the physiological variables during the cycling efficiency test post-training compared to the S and SUL protocols, even though they showed similar reductions in mean MVC torque. Furthermore, the difference did not seem to be the result of muscle damage, as outlined earlier in this discussion. The reduced interference may be related to the movement pattern of the hill cycling being the same as that of the sub-maximal cycling exercise compared to the notably different movement pattern of the conventional strength training protocols. During cycling the muscle contractions are intermittent, as one leg provides force, the other recovers and the percentage contribution of the various leg muscles to force production changes as the pedal revolution is performed (Citterio & Agostoni, 1984; Ericson et al., 1985). In contrast, during weight training exercises such as leg-press or leg-extension, tension is generated in the same muscles throughout the movement (Lambert & Flynn, 2002). Furthermore, the movement patterns of the leg-press, leg-extension and leg-curl exercises are in a straight line (raising and lowering) (Stauber, 1989) compared to the cycling action, where the limb travels in a circular motion (Burke, 1995; Citterio & Agostoni, 1984; Ericson et al., 1985). This difference in movement pattern may have hindered the organisation of efficient motor unit recruitment patterns in the efficiency tests following the conventional strength training protocols, as suggested by Chromiak and Mulvaney (1990) in their review of the effects of combined strength and endurance training on strength development. This suggestion was also proposed by Gleeson et al (1995) after their observation of an increased physiological cost during sub-maximal cycling exercise after a bout of bench stepping compared to up-hill walking.

The above discussion showed how changes in motor unit recruitment might influence endurance cycling. However, the same might also apply to strength training completed after endurance training as highlighted by the 20% increase in post-training BL concentration for
the W session of the CW sequence in Study 2 even though there was minimal change in the MVC torque following the C session. Similar alterations in motor unit recruitment patterns may have also influenced the W session of the CW sequence in Study 3. Unfortunately this is only speculation and as such further research is necessary to determine the influence that alternative (hill cycling) and conventional strength training sessions may have on alterations in motor unit recruitment patterns during subsequent endurance exercise as well as the reverse scenario of endurance training prior to strength training. Further research is also required to determine if such alterations in motor unit recruitment patterns during concurrent training sessions performed on a single day are a factor in the long-term development of strength and/or endurance.

7.3.2.2 Influence of Subject’s Training Age and/or VO$_2$ max

A factor that appears to have influenced the level of recovery following the strength training sessions as well as cycling efficiency in the current series of studies was the training age and/or VO$_2$ max of the subjects. The subjects were regularly undertaking strength and endurance training prior to each of the studies and as such may have adapted to the stress of regular training more than lesser trained or untrained subjects (Melby et al., 1992; Thornton & Potteiger, 2002). Apart from the repeated bout effect, evidence that this may have been the case was provided in the observation from the efficiency test results that those subjects with a greater training age showed reduced levels of physiological stress than those of a lesser training age, similar to that found for the MVC trials. This observation along with the large variation in efficiency between the subjects, promoted the completion of a correlation analysis between GE and NE and VO$_2$ max in Study 1 to determine if VO$_2$ max might have been an influencing factor in the efficiency test results. The statistical analysis confirmed the presence of such a relationship, as a strong positive correlation was found between GE and NE and VO$_2$ max for all strength protocols including the CE test. This finding conflicts with previous findings in this area, which have found no correlation (Nickleberry & Brooks, 1996; Stuart, Howley, Gladden, & Cox, 1981) or a weak correlation at best (Moseley & Jeukendrup, 2001). An interesting finding of the analysis was that the relationship weakened as the intensity of the exercise increased from the 20 to 60% workloads but not for the SUL and S protocols which, maintained relatively strong correlations ($r = .77-.85$) for both GE and NE compared to the other protocols ($r = .17-.59$).
The high correlation between GE and NE and VO₂ max at low workloads, suggests that those subjects with a greater VO₂ max were more efficient at lower workloads than those with a lower aerobic capacity. This relationship may be attributed to a difference in muscle fibre type in the exercising muscles between the subjects. Even though muscle fibre composition analysis was not carried out in this study, it has been reported that those subjects with a greater proportion of slow twitch fibres are more efficient at low power outputs (Suzuki, 1979). Further support for this suggestion comes from studies that have shown GE to be well correlated with the percentage of slow twitch fibres present in the exercising muscles (Coyle et al., 1992; Horowitz et al., 1994).

Due to the relationship between GE and NE and VO₂ max still being very strong for the SUL and S protocols compared to the other protocols at the highest workload suggests that there was an interaction between the type of training protocol and the VO₂ max of the subjects. This finding suggests that the oxidative capacity of the exercising muscles may be important in determining the extent of fatigue following high-intensity strength training. Suzuki (1979) suggested that those with a greater proportion of fast twitch fibres are more efficient at high power outputs. However, this may not hold true for exercise completed after strength training and may be due to the fatigue pattern within the muscle. A number of previous investigations (Kanehisa et al., 1997; Komi & Tesch, 1979; Thorstensson & Karlsson, 1976) have shown that those individuals with a greater percentage of fast twitch fibres, whilst able to generate a greater level of muscular force, are also more susceptible to fatigue during repeated maximal dynamic contractions. Such a finding has been attributed to greater disruption in the contractile components of the fast twitch fibres resulting in a slower strength restoration following exercise (Fridén, Seger, Sjöström, & Ekblom, 1983; Nilsson, Tesch, & Thorstensson, 1977). Consequently, it is possible that those subjects that had a greater percentage of fast twitch fibres may have suffered a higher level of fatigue following the SUL and S protocols than those subjects with a higher VO₂ max and a greater percentage of slow twitch fibres.

In addition, it has been previously suggested that endurance training adaptations such as increased muscle blood flow enhancing lactate exchange from active muscles to removal sites, may enhance the ability of endurance-trained athletes to recover faster from exhaustive exercise than sprint trained athletes (Taoutaou et al., 1996). Therefore, it is possible that the relationship between cycling efficiency and VO₂ max at the high workload may also be
related to the greater level of endurance adaptations such as increased muscle blood flow due to increased capillary density within the muscle (Terjung, 1995). These adaptations allowing a greater level of oxygen extraction and waste exchange due to a greater contact surface area between capillaries and muscle fibres (Terjung, 1995), thereby enabling a faster recovery from the high-intensity strength training protocols.

This possible relationship between muscle fibre type and fatigue following the strength training sessions may partly explain why the H protocol showed similar reductions in MVC torque similar to that of the S and SUL protocols in Study 1. The correlation between GE and NE and the 60% of VO₂ max workload in Study 1 support this in part because after the S and SUL protocols, the next highest correlation was for the H protocol (r=.59). Whilst this was not a strong correlation, it was consistent with the observation from the MVC trials that those with a greater training age showed less fatigue than those with a lesser training age. This finding together with the fact that CK and a number of the efficiency test variables responded similarly following the strength training sessions for the SE and H protocols, also suggests that other unidentified factors are contributing to fatigue following the H protocol. These unidentified factors may also explain the reduced physiological cost during the efficiency test following the strength training sessions compared to the other protocols.

The training age and/or VO₂ max of the subjects may have also been a contributing factor to the large variability found for the hormonal and gene expression responses in Study 3. In a study of the magnitude and time course of changes in muscle protein synthesis after a single bout of strength training which resulted in wide inter-individual differences, Chesley et al (1992) suggested the source of the variation was possibly due to the differences in training history, muscle fibre composition and/or the degree of muscle damage sustained during exercise. Similar reports have also been proposed after finding a strong negative correlation between LPL activity and the amount of training before a ski race as well as a relationship between the amount of ski training and VO₂ max and performance time (Lithell et al., 1979). Bishop et al (1999) also suggested that the training status of the individual was the reason why trained subjects may not respond to concurrent training regimes similar to those of untrained subjects (Kraemer et al., 1995; Nelson et al., 1990; Sale et al., 1990a) because of a limited potential for further adaptation to take place due to a already existing high levels of adaptation.
In view of the above findings, more research is required to examine the possible relationship between training age and/or VO2 max and recovery dynamics in concurrent training regimes as well as the influence on strength and endurance adaptations.

7.4. Influence of Prior Exercise on the Perception of Effort

A common finding of the C training sessions in Study 2 and 3 as well as the efficiency tests following the various strength training sessions in Study 1 was the increased perception of effort. Whilst the influence of the prior strength training sessions on RPE was not significant in all studies, it was particularly noticeable for the C session of the WC sequence in Study 3 where RPE averaged 10% higher over the duration of the session than that found for the CW sequence.

Ratings of perceived exertion have been attributed to input from both central and peripheral sources (Cafarelli, 1977; Ekblom & Goldberg, 1971; Noble et al., 1973). Central input refers to cardiorespiratory responses whilst peripheral input refers to local factors associated with feelings of strain in the working muscles and joints of the exercising limb (Cafarelli, 1977; Ekblom & Goldberg, 1971; Pandolf & Noble, 1973). Given that the majority of subjects in the three present studies showed increased physiological responses for the C sessions completed after the strength training sessions along with a possible increased level of strain from a greater amount of exercising muscle mass due to muscle fatigue (Sale, 1987; Wilson, 1994a), it is possible that these factors contributed to the increased perception of effort during the C sessions and efficiency tests. Similar response patterns between the physiological variables and RPE have been reported previously during exercise (Cafarelli, 1977; Ekblom & Goldberg, 1971). The connection between central and peripheral sources and RPE may also explain why the subjects’ RPE showed similar workload sensitivity, as found for some of the other physiological variables in Study 1.

The majority of subjects in Study 3 also reported an increased psychological stress due to feeling a higher level of anxiety both before and during the C session of the WC sequence. They attributed this increased anxiety level to being unsure that they could maintain the required intensity for the duration of the exercise, as they felt they had not recovered sufficiently from the prior W session. This anticipation may be the reason why small but non-significant increases in testosterone were found at the commencement of the C session for the
WC sequence. A similar anticipatory testosterone response has been reported previously in subjects prior to the completion of varying intensities of treadmill exercise and attributed to the subjects’ perception of forthcoming work (Wilkerson, Horvath, & Gutin, 1980). The increased level of anxiety as reported by the subjects may have also contributed to the increased cortisol response found for the C session of the WC sequence compared to the CW sequence, as the cortisol response is affected by psychological stress (Chattoraj & Watts, 1986).

7.5. Residual Fatigue as a Possible Source of Long-Term Interference in Concurrent Training Adaptation

The current series of studies investigated a number of the acute residual fatigue mechanisms (muscle damage, neuromuscular fatigue and muscle glycogen depletion) thought to contribute to the interference of strength and/or endurance responses and hence adaptations in concurrent training regimes (Chromiak & Mulvaney, 1990; Leveritt et al., 1999). The issue of whether these acute mechanisms may influence the long-term development of strength and/or endurance has not been addressed as yet. The discussion of the abovementioned mechanisms has been combined under the heading residual fatigue however muscle damage is also discussed separately because of issues inherent to that mechanism.

7.5.1. Muscle Damage

Whilst some form of muscle damage may have contributed to the reduction in force generating capacity and increased physiological cost during the efficiency tests following the strength training sessions in Study 1 and 2 as well as the C sessions in Study 2 and 3, the level of muscle damage as shown by the CK response for the first two studies was low. The levels reached by the majority of the subjects following the training protocols and sequences were only slightly above the upper limit of 175 U.L\(^{-1}\) observed at rest for healthy subjects (Rose, 1995) and is consistent with low post-training CK levels (109-800 U.L\(^{-1}\)) found for other trained athletes (Dolezal et al., 2000; Robinson et al., 1982). It is possible that the time course of the CK response may have been a factor that influenced the results in that research has shown that the CK response can peak between 24 and 120 hours post-training (Clarkson & Tremblay, 1988; Dolezal et al., 2000; Tokuda & Otsuji, 1990). However, the more likely contributing factor was that the subjects were well trained in both strength and endurance
exercise. Previously it has been found that following repeated exercise bouts, termed the ‘repeated bout effect’ (Hortobágyi & Denahan, 1989), there is a profound reduction in the elevation of plasma CK (Ebbeling & Clarkson, 1989; Evans et al., 1986; Jamurtas et al., 2000; McHugh, 1999; Newham et al., 1987). The reduced CK levels following repeated bouts of exercise attributed to connective tissue undergoing structural changes due to forced lengthening making muscle fibres resistant to further damage (Appell et al., 1992; Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1989; Kuipers, 1994). Consequently, because in the present study all subjects performed weight training at least two times per week for many months prior to the experiment, it is possible that they had become more resistant to the muscular damage associated with strength training.

The low CK responses following the training sessions in Study 1 and 2 support the suggestion of Leveritt et al. (1999) that muscle damage is an unlikely source of long-term interference in strength development because of the repeated bout effect and the fact that the degree of muscle damage following strength training is generally greater than that following endurance training. The present CK findings also suggest that the type of endurance activity undertaken in a concurrent training regime may contribute to the compatibility of endurance and strength training sessions. The possible compatibility of cycling and strength training was highlighted by the results of the four subjects that completed Experiment 1B and who also took part in Study 2. These four subjects showed very similar mean CK responses for the SUL protocol (201 ± 17 U.L⁻¹) and the WC (205 ± 73 U.L⁻¹) and CW (213 ± 64 U.L⁻¹) sequences. Even though a period of a 2-3 months had passed between the completion of Study 1 and Study 2, the results of these four subjects indicated that the completion of a one-hour cycling session at 60% of VO₂ max, either before or after the W session, did not increase the level of damage incurred by subjects to any notable level beyond that incurred following a single W training session. This suggests that moderate intensity endurance exercise using concentric contractions only, performed prior to or following a bout of high-intensity strength training, does not increase the level of damage incurred following a single strength training session. Furthermore, there is some evidence to suggest that there may be a relationship between the type of strength training (isotonic) and the mode of endurance training (running) in determining the interference in strength development (Leveritt et al., 1999). Consequently, the current finding is important because the type of endurance training undertaken in a concurrent training regime may be a key component in determining the level of muscle damage and
subsequent fatigue incurred post-training and hence the degree of interference in strength and/or endurance development.

Unfortunately there have been no previous investigations of the degree of muscle damage that occurs during concurrent training regimes. Therefore there is insufficient evidence to make a conclusive argument to totally disregard this mechanism as a factor in the interference of strength and endurance development. Further research utilising various combinations of strength and endurance exercise (cycling, running, rowing etc) with different intensities, durations and training sequences, need to be undertaken. In addition, more direct methods of measuring muscle damage such as electron microscopy need to be utilised in order to enable a more accurate assessment of the degree of damage to muscle ultrastructure following bouts of concurrent training as well as the effect that repeated days of concurrent training may have on muscle repair processes.

7.5.2. Residual Fatigue

The hypothesis of residual fatigue impeding the development of strength during a concurrent training programme due to a reduction in muscle tension generation was proposed by Craig et al (1991), who observed greater increases in leg-strength and thigh diameter for a S trained group compared to a COMB group after 10 weeks of concurrent training. Whilst a number of concurrent training studies have shown similar findings (Dolezal & Potteiger, 1998; Dudley & Djamil, 1985; Hortobágyi et al., 1991; Sale et al., 1990b), the question still remains is acute residual fatigue from a prior training session the possible source of long-term interference in concurrent training adaptation? The findings of reduced force capabilities and elevated physiological responses in the training sessions completed second in the training sequence in the current series of studies provides evidence that such a mechanism could be a contributing factor to both strength and endurance development in concurrent training programmes.

It has previously been reported that prolonged (Lepers et al., 2000; Sherman et al., 1984) or shorter high-intensity endurance exercise (Bentley, 1998; Bentley et al., 1998) can significantly affect force generation capacity post-exercise. Whilst the present findings of Study 2 were not consistent with these findings, as outlined in the discussion for Study 2, there was an increased physiological cost of exercise, as indicated by the elevated BL and HR responses found for the W sessions of the CW sequence in Study 2 and 3, respectively. Even
though, the subjects completed the exercises of the W session for the CW sequence, yet with some difficulty, it is possible that the level of tension in individual motor units was not the same as when the same sessions were completed first in the training sequence. The level of tension in individual motor units may be changed due to altered motor unit recruitment patterns (Chromiak & Mulvaney, 1990) and/or a greater contribution from other muscle groups in response to muscle fatigue or damage (Coyle, 1995; Perrin, 1993), or possibly due to selective glycogen depletion of muscle fibres (Gollnick, Armstrong, Sembrowich et al., 1973; Gollnick et al., 1974; Pascoe & Gladden, 1996).

The sequence of completing the strength and endurance training sessions in Study 3 also affected the responses of the hormones, testosterone and cortisol as well as the expression of a number of the genes associated with metabolic and muscle growth functions. Therefore, it is conceivable that it might not be just the level of tension per se that is responsible for the reduction in strength development reported previously in the literature but the culmination of a reduction in muscle tension and increased stress associated with trying to overcome this deficit. The increased stress altering hormonal and gene expression responses post-exercise associated with muscle growth and function may reduce the level of strength development over time.

A similar suggestion of reduced endurance development may be the consequence of residual fatigue from the prior strength training session. This was highlighted in the present series of studies by the increased physiological cost and reduced GE and NE during the C sessions and efficiency tests following the strength training sessions. The influence of this increased physiological cost and altered efficiency during the endurance training sessions is that there could be a decrease in performance capacity of the endurance exercise component due to a reduction in the intensity and/or the duration of the exercise (Bahr, 1992). A reduction in exercise intensity because of an increased level of fatigue in the exercising muscles inhibiting the ability of the individual to achieve high power outputs as well as the possible increased rate of metabolite accumulation such as BL, resulting in premature cessation of exercise prior to maximal exercise intensities being reached (Gleeson et al., 1995). The reduction in exercise duration because of the elevated resting and exercise metabolism causing additional energy expenditure (Bahr, 1992) and a greater rate of muscle glycogen depletion contributing to an earlier onset of fatigue (Gleeson et al., 1995). The implications of these increased physiological responses following a bout of strength training have not been studied in
previous concurrent training investigations. However, the current findings (e.g. increased BL response and change in substrate utilisation during the C session of the WC sequence in Study 2 and 3, respectively) support the suggestion that a reduced exercise intensity and/or duration in endurance exercise may lead to a reduced level of adaptation compared to when endurance exercise is performed as a single mode of training (Leveritt et al., 1999).

Whilst it could be argued that an increased stress to maintain force or power output during the W and C sessions of the CW and WC sequences and the subsequent increased physiological cost could improve adaptation by increasing the effectiveness of the exercise stimulus, the opposite could also be true. That is, that the increased physiological cost could increase the stress response of the training stimulus beyond what would normally be evoked by a single session of training resulting in a negative response. One such response could be an increase in the concentration of the hormone cortisol, which has been shown to be elevated in times of physical stress (Chattoraj & Watts, 1986). The notable increase in the cortisol response and subsequent reduction in the T/C ratio for the WC sequence compared to the CW sequence in Study 3 supports this line of reasoning. Therefore, it is conceivable that concurrent training regimes may over stress an individual’s normal response mechanisms producing a negative environment similar to that found in overtrained athletes, resulting in a reduced level of adaptation over time (Hooper & Mackinnon, 1995; Newsholme et al., 1992). However, this response may only be associated with the training session that occurred second in the training sequence, as highlighted by the elevated responses of the majority of the variables measured during the second training session in the current series of studies. Therefore, the negative environment may not be in response to overtraining due to the total volume of concurrent training as originally proposed for the impedance of strength and/or endurance development (Hickson, 1980) but to the cumulative effect of the session with the increased stress. Such a scenario also gives support to the suggestion by Leveritt et al (1999) that the concept of overtraining being responsible for impeded adaptation was based on the assumption that strength and endurance training had similar overtraining thresholds but this may not be the case.

In association with the sequence of training and the increased physiological cost of performing endurance exercise following a strength training session, it is worth noting that two previous studies (Dolezal & Potteiger, 1998; Nelson et al., 1990) reported a lower level of aerobic adaptation for a COMB group compared to an E group. Both studies used a
percentage of maximum heart to set the workload intensity for the aerobic training component of the study. The aerobic training in these studies was completed after the strength training component of the concurrent training programme. Based on the elevated HR response for the C session of the WC sequence in Study 2 and 3, it is conceivable that these previous studies which completed the aerobic training shortly after the resistance training, using untrained subjects, may have ‘inadvertently’ under-trained the subjects. This under-training occurring because of a reduced intensity (power output) for a given HR compared to those subjects that completed the aerobic training without any proceeding exercise. Whilst, this is only speculation and does not account for those studies that reported reduced aerobic capacity for a COMB group but did not use HR as the criteria for setting exercise intensity (Gravelle & Blessing, 2000; Kraemer et al., 1995), it does highlight the problem of completing concurrent training sessions within a short period of time, prior to the physiological status of the individual returning to pre-exercise levels.

In summary, the findings of the present series of studies showed that residual fatigue is a factor in the completion of concurrent training sessions by reducing force generating capacity and increasing the physiological cost of those sessions completed second in the training sequence. The present results also suggest the increased physiological cost may be the result of one or combination of a number of factors including possible damage to muscle contractile structures, neuromuscular fatigue and/or alterations in motor unit recruitment patterns. The findings also highlight the extent of interference of a prior training session on subsequent training sessions is dependent not only on the intensity and types of training performed, but the scheduling of training sessions and training age and/or VO2 max of the subjects. These factors contributing to long-term interference in strength and endurance development in concurrent training regimes remains to be investigated.
CHAPTER 8

Summary and Conclusion

8.1. Introduction

In this thesis, several methodological issues of concurrent training regimes were investigated including the intensity, duration and mode of strength training as well as the sequence in which concurrent training sessions are completed and their effect on cycling efficiency and force generating capacity. In addition, a number of mechanisms that may be responsible for the interference or impedance of strength and/or endurance adaptation reported in the literature were examined including changes in muscle glycogen content and hormone concentrations and alterations in the expression of genes associated with skeletal muscle growth and metabolic functions. With reference to the aims of this project, the major findings of this thesis are outlined below.

8.2. Comparison of the Intensity of Strength Training Sessions when Equated for Work

In Study 1, a greater reduction in peak and mean MVC torque was found post-training for the high-intensity S protocol compared to the low-intensity SE protocol when equated for work, suggesting a greater level of fatigue. The difference in the recovery of force generating capacity between the S and SE protocols was attributed to some form of peripheral fatigue, possibly due to a difference in the level of muscle damage, as suggested by a higher CK response found for the S protocol compared to the SE protocol. The S protocol also exhibited a higher physiological stress during the cycling efficiency test than the SE protocol as suggested by the significantly higher HR response and small but consistently higher responses for a number of the other physiological variables including BL, RR, V_E, VO_2 and TT. It was concluded that the high-intensity S protocol had a greater residual physiological effect on muscle force generating capacity and the physiological cost of cycling than the low-intensity SE protocol when equated for work volume.
8.3. Comparison of the Duration of Strength Training Sessions

The longer duration (60-minute) SUL protocol in Study 1 – Experiment 1B, which incorporated exercises for both upper and lower body muscle groups, produced a similar significant reduction in mean MVC torque and superimposed and control twitch torque (an indication of peripheral fatigue) as the shorter duration (30-minute) S protocol, which involved use of only the lower body muscle groups. These similarities suggested that the extension of the duration of the training session via the inclusion of additional exercises that used muscle groups other than those used during the S protocol was not a factor in the recovery of force generating capacity post-training. In contrast, the SUL protocol produced a greater physiological stress during the cycling efficiency tests than the S protocol as indicated by the higher cardiorespiratory responses, BL concentration, TT and greater reduction in GBM, GE and NE compared to the CE test. This finding may be due to the greater volume of exercising muscle mass for the SUL protocol compared to the S protocol. It was concluded that the longer duration SUL protocol had a greater residual physiological effect on cycling performance than the shorter duration S protocol of similar intensity due to a greater systemic stress but a similar effect on the recovery of force generating capacity due to a similar level of localised lower body fatigue.

8.4. Comparison of the Modes of Strength Training

In Study 1, the H protocol which involved only concentric contractions and twice as much work as the S and SE protocols, produced a similar significant reduction in mean MVC torque as the S and SUL protocols which involved eccentric and concentric contractions. Minimal change was found for the SE protocol. The source of the residual fatigue was suggested as peripheral, indicated by a reduction in both the control and superimposed twitch responses. However, the similarity between the H protocol and the S and the SUL protocols appeared to be due to reasons other than muscle damage as suggested by the difference in CK responses found between the protocols. In contrast, the SUL and S training protocols exhibited a higher physiological stress than the SE and H protocols compared to the CE test, a difference that appeared to be related to the intensity and duration of the prior exercise and not the mode of training. The above findings suggest that the mechanisms contributing to the reduction in force generating capacity and increased physiological cost of cycling post-training may be different for the S and SUL protocols compared to the H protocol. It was concluded that the
mode of strength training is an influencing factor in the recovery of force generating capacity and on subsequent cycling exercise performed post-training. Furthermore, it appears that conventional high-intensity strength training may have a greater physiological effect than hill cycling strength training.

8.5. Effects of the Sequence of Completing Strength and Endurance Training Sessions

(i) The physiological stress of the second training session compared to when the training sessions were completed first

The results of Study 2 and 3 both showed an increased physiological stress for the W session of the CW sequence compared to the WC sequence. This was highlighted by a higher BL concentration post-training in Study 2 and a significantly elevated mean HR response in Study 3. Similarly, elevated cardiorespiratory responses and changes in GE and NE were found for the C session of the WC sequence compared to the CW sequence in both Study 2 and 3. The notably higher respiratory responses, downward shift in RER and subsequent greater reduction in GE and NE during the C session of the WC sequence in Study 3 compared to Study 2 also suggested that the physiological stress of the second session was influenced by the type of training protocol of the prior W session. In addition, that the duration of the C session was a contributing factor with an increase in the physiological cost of exercise as the C session progressed. The increased physiological stress during both the W and C sessions in the CW and WC sequences, respectively was attributed to:

- an elevated resting metabolism prior to the commencement of the second training session, and
- the residual fatigue of the prior training session requiring the recruitment of a greater percentage of fast twitch fibres and/or inefficient motor units to maintain power output.

It was concluded that there was an increased physiological stress during those sessions completed second in the training sequence compared to when the same training sessions were completed first, irrespective of the type of training.
(ii) The recovery dynamics of both force generating capacity and physiological parameters post the second training session

A significant reduction in mean torque was found for the WC sequence compared to the CW sequence in Study 2, which showed only half the deterioration in force generating capacity following the second training session. Whilst some form of peripheral fatigue was identified as contributing to the result, the fact that the completion of the W session prior to the C session attenuated the fatigue normally found following the C session conducted without the preceding W session suggested that the reduction in force generating capacity during and following the two training sequences was due to different fatigue mechanisms or that the fatigue mechanisms that contributed to the first training session were compounded by the second training session. Higher physiological responses were also found during the efficiency test following the WC sequence compared to the CW sequence. This finding suggested that the recovery dynamics were different following the WC and CW training sequences. It was concluded that the recovery of force generating capacity and physiological parameters following the training sessions were dependent on the sequence of training, with the sequence WC requiring a greater recovery period than the sequence CW to return to the pre-exercise state.

8.6. The Effects of the Sequence of Completing Strength and Endurance Training Sessions on the Same Day

(i) The level of skeletal muscle glycogen depletion and recovery, during and following the training sessions

No significant change in the pattern of glycogen depletion for the W or C session was found in either sequence. These findings suggest that the completion of strength and endurance training on the same day did not directly affect skeletal muscle glycogen levels either during or following exercise. However, the present findings suggest that indirectly the prior W session influenced the type of substrate utilised during the C session of the WC sequence by causing a shift from carbohydrate to lipid oxidation, thereby providing a muscle glycogen sparing response. The results also suggest that even though the W session of the CW was completed in a depleted glycogen state compared to the WC sequence, due to a significant reduction in muscle glycogen content following the C session similar to that found for the CW
sequence, it did not prevent the completion of the high-intensity W session. Both the WC and CW sequences recovered to 73 and 81% of pre-training day levels 25 hours following the W session, respectively. Whilst the results suggested that reduced muscle glycogen levels were not a factor in the performance of the concurrent training sessions, the fact that the level of muscle glycogen was still below pre-exercise levels the day following exercise for both training sequences, may have implications for the completion of subsequent training sessions, particularly if that session was endurance-orientated. It was concluded that the sequence of training did not directly affect the level of muscle glycogen depletion following the training sessions but indirectly affected the choice of substrate during the C session of the WC sequence. Further, it was concluded that low muscle glycogen content prior to the commencement of W session does not prevent the completion of the session and that a period greater than 24 hours was required to fully restore muscle glycogen content after bouts of prolonged cycling exercise accompanied by strength training either prior to or following.

(ii) The hormonal responses of testosterone and cortisol compared to single mode training sessions

Temporal increases in testosterone and cortisol were found following the second training session for both the WC and CW sequence days in Study 3 compared to the control days. These findings suggested that the completion of a second training session altered the hormonal response pattern following single mode training sessions. It was also found that the WC sequence stimulated the hormonal system to a greater extent than the CW sequence as evidenced by a greater temporal increase in testosterone and cortisol in the hours following the second training session, resulting in an altered T/C ratio. The WC sequence produced a lower T/C ratio than the CW sequence, suggesting a more catabolic state for the WC sequence. The greater hormonal response and lowered T/C ratio following the second training session of the WC sequence was attributed to the increased physiological stress of the C session stemming from a high level of residual fatigue from the prior W session including the level or rate of muscle glycogen depletion in the exercising muscles. It was concluded that the completion of a second bout of training, irrespective of whether it was strength- or endurance-orientated, altered the testosterone and cortisol responses compared to the single mode training sessions. Further, it was concluded that the sequence in which the concurrent training sessions were completed was an influential factor in determining hormonal responses post-
training and not just the inclusion of endurance training in a strength training regime or vice versa.

(iii) The level of expression of selected genes associated with skeletal muscle growth and metabolism

Of the six genes associated with muscle growth that were examined in Study 3 (MHCI, MHCIIa, MHCIIx, IGF1 & 2, MyoD and myogenin), only the MRF genes MyoD and myogenin were affected by the training sequences. This appears to be due to these genes responding more to the CW sequence than the WC sequence, especially at the 25 hour post W time point where significant ~5-6 fold increases in mRNA content were found. In contrast, the genes PDK4, HKII, PGC1 and LPL which are associated with metabolic functions responded more to the WC sequence than the CW sequence. The remaining genes associated with muscle metabolic function (GYS, UCP3, LDH-M, LDH-H and CPT1) did not respond more to one training sequence than another. The responses were generally greater following the second training session for both groups of genes which suggested that the type of training completed second in the training sequence may be important in determining the responses of the selected genes. This finding also suggested that the factors governing the responses of the two groups of genes may have been different, with the genes associated with muscle growth responding to the intensity of muscle contraction or contractile activity pattern during the W session, whilst the genes associated with metabolic function may respond to the choice of substrate being utilised during the C session. It was concluded that the level of expression of some genes associated with skeletal muscle growth and metabolic functions were altered by the sequence in which the concurrent training sessions were completed. In addition, it was concluded that the sequence in which the concurrent training sessions were completed was an important factor in determining the expression of genes post-training similar to that found for the hormonal responses.
8.7. Practical Implications

The findings from the current series of studies highlighted a number of practical implications for coaches and athletes involved with concurrent strength and endurance training programmes. These include: (i) the modes and types of strength training used for concurrent training; (ii) the recovery period between strength and endurance training sessions; (iii) the order that strength and endurance training sessions are completed; (iv) the increased physiological stress during training sessions completed after prior exercise; and (v) the influence of the training status of the athlete.

(i) The modes and types of strength training used for concurrent training

The findings of Study 1 suggest that the H protocol did not affect the physiological cost of cycling during the efficiency test to the same extent as the high-intensity SUL or S protocols, despite twice as much mechanical work being completed. It is possible that hill cycling may produce less interference of endurance adaptation than that reported for conventional strength training (Dolezal & Potteiger, 1998; Kraemer et al., 1995; Nelson et al., 1990). Further, because of the specificity of hill cycle strength to cycling performance, it is suggested that strength gained through this mode of training may have a greater transference to cycling performance than conventional strength training methods. This suggestion is supported by previous research that has investigated the effects of conventional strength training on sporting performance and failed to show improvement in endurance performance (Bishop & Jenkins, 1996; Bishop et al., 1999; Tanaka et al., 1993). Bishop et al (1999) suggested that the volume of resistance training might be a factor however this is yet to be established, whilst Tanaka et al (1993) suggested that the lack of positive transfer between the dry-land strength improvements and swimming performance was due to conventional strength training not being specific to the technical requirements of the swim stroke. However, in a study by Toussaint and Vervoorn (1990), it was found that in-water resistance training improved a swimmers velocity over distances up to 200 metres as well as improving force and distance per stroke.

The results of Study 1 also suggested that short duration (30-minute) sessions had less of an effect than a high-intensity long duration (60-minute) session on subsequent cycling
performance. The differences between the S and SUL strength training protocols for the various physiological parameters were not significant in all cases. However, the findings suggested that there may be less residual fatigue and hence less physiological disturbance from a strength training session to a subsequent endurance training session if the strength training session is short in duration and if adequate recovery time is provided between sessions (> three hours). Further, the evidence from previous research comparing various strength training protocols has identified that the greatest disturbances to homeostasis occur from protocols designed to develop hypertrophy (Schuenke et al., 2002). Taken together with the present findings, this suggests that the recovery period between strength and endurance training sessions in a concurrent training regime may need to be varied according to the type of strength training being performed. It also suggests that the use of split routines for athletes wishing to perform strength training in conjunction with their endurance training routine may be of benefit. This would enable various muscle groups of the body to be trained for a short period of time, whilst minimising the residual effect of one session to the next within the same day.

(ii) The recovery period between strength and endurance training sessions

The increased level of fatigue, cardiorespiratory and hormonal responses of the second training session following either prior strength or endurance training suggests that a greater level of physiological stress was experienced by subjects during these subsequent sessions. The practical implication of this is that a longer recovery period greater than three hours is required for complete recovery between training sessions of similar type and intensity as those used in the current studies. This may help avoid any adverse residual effect from one training session to the next. This increased fatigue, physiological stress and/or altered physiological responses may interfere with the development of strength and/or endurance development in subsequent training sessions. Therefore, those athletes that complete two or more training sessions in a day, with three hours or less of recovery between sessions may limit their training potential either by reducing the training intensity and/or duration due to the negative residual effects of the prior training bout. Subsequently they may benefit from either increasing the recovery time between training sessions or limiting the number of training sessions completed in a single day to two, with one session completed early and the other late in the day, in order to provide sufficient rest between the training stimuli.
The present results also highlighted the benefit that may be gained from ensuring proper physiological and psychological recovery techniques are employed between training sessions to limit the possible negative affects from prior training sessions. One of the most important recovery aspects, suggested by the muscle glycogen findings of the present studies, was the ingestion of carbohydrates immediately following exercise to aid the recovery of muscle glycogen levels, particularly after exhausting endurance exercise and/or when training is to be completed over successive days. In addition, appropriate (amount and timing of ingestion) pre- and during exercise dietary plans should be utilised to reduce the degree of muscle glycogen depletion and the possible limiting affects of low muscle glycogen content on exercising performance.

(iii) The order that strength and endurance training sessions are completed

The results of Study 2 showed that the order that the strength and endurance training sessions are completed during a concurrent training regime is important in determining the rate of recovery of force generating capacity, with the WC sequence showing slower recovery than the CW sequence three hours following the second training session. This finding suggests that completing training in the order of CW may reduce the time required for recovery compared to the WC sequence. However, because of the residual fatigue factor from the first training session on subsequent training sessions as demonstrated in the three studies, the order of CW may not suit all concurrent training programmes. In view of this, the guideline that may be most beneficial is the priority concept (Fleck & Kraemer, 1997) which suggests that the physiological component considered most important for the respective sport be trained first. Therefore, for a cyclist whose main aim is to increase aerobic power, then the CW sequence would be most beneficial. This idea was also proposed by Leveritt and Abernethy (1999) after finding that an acute bout of high-intensity endurance training reduced force generating capacity post-exercise. It should also be mentioned that the priority given to each training session would more than likely change throughout the training year as the emphasis of various components of training changes to meet the goals of that particular training phase. In association with priority training it may also be beneficial for those sports such as distance cycling and running, that require the completion of long sessions of aerobic training, to complete these long training sessions on days when there are no other training commitments, so as to avoid the possible impingement either from or on the other training sessions performed on the same day.
(iv) The increased physiological cost during training sessions completed after prior exercise

There is the potential for prior training sessions to increase the physiological cost of subsequent training sessions when training sessions are completed within close proximity to each other. Thus, thought must be given to the method employed to set exercise intensity. For example, an elevated heart rate following the first training session, could mean that the intensity of subsequent training sessions are conducted below the designated level and thereby lowering the rate of adaptation over time. However, the opposite is also possible. That is, if the increased physiological cost of training is not considered (increased dietary requirements, rest periods etc) and monitored, then there is the potential for a state of overtraining to occur (Bahr, 1992).

In addition to the increased physiological cost of completing a C session after a W session, consideration has to be given to the possible increased level of anxiety or psychological stress incurred due to a feeling of insufficient recovery, as indicated by the subjects in the Study 3 prior to commencing the three-hour C session. This psychological aspect of concurrent training may limit the performance of training sessions completed second in the training sequence, even if the physiological mechanisms have returned to normal. This may be done by reducing the intensity and/or duration of the training session, resulting in a lower exercise stimulus and physiological response and hence a lower level of adaptation.

(v) The influence of the training status of the athlete

A number of the variables measured during the studies (e.g. CK activity, physiological responses during the efficiency test including GE and NE) showed that they were affected by the training age and/or VO2 max of the subjects, which indicates that the level of training that the athlete is at must also be taken into account when designing a concurrent training programme. The findings suggest that athletes with a greater training age and/or VO2 max showed lesser disturbance to strength and reduced physiological cost during exercise following strength training than those with a lesser training age or VO2 max. Therefore, concurrent training programmes involving less well-trained athletes may require a greater recovery time between training sessions or a slower progression of strength training intensity and/or duration than more highly-trained athletes.
8.8. Directions for Future Research

Whilst the current series of studies have contributed to a greater understanding of the compatibility of concurrent training sessions, a number of areas requiring further research were also identified. These include:

(a) **The acute effect of strength training on other modes of endurance training**

To the investigators knowledge, the present thesis is the first to investigate the acute effects of strength training on endurance cycling performance on the same day. The current findings suggested that cycling performance is affected by a prior bout of strength training with respect to the ability to generate force as well as a greater physiological cost of exercise. However, further research into the effects of various types (high- and low-intensity) and durations of strength training on other modes of endurance exercise (e.g. running, swimming and rowing) is required. Furthermore, the effect of different training sequences is required to determine if the mode of exercise is an influencing factor in the responses to concurrent training sessions and possibly the impedance of adaptation. In conjunction with the above, higher intensity endurance exercise than that used in the current studies needs to be included to determine if the intensity of the endurance training sessions is also a factor in the responses found post-exercise.

(b) **Comparison of modes of strength training and conventional strength training on the transference to improved endurance performance**

The present results of the H training protocol in Study 1 indicated less residual affect on the physiological cost of cycling three hours post-training than the high-intensity S protocol even though twice as much mechanical work was completed. This finding may have been due to the specificity of hill cycling strength training having a greater transference to subsequent cycling performance than conventional strength training. However, there is no evidence available to support this suggestion. Consequently, further research is required into the strength gains achieved through sport-specific strength training such as hill cycling, resisted swimming and hill running compared to conventional strength training techniques and how these strength gains relate to improvements in sporting performance. Furthermore, it remains to be determined how the strength adaptations gained through sport-specific strength training
are affected by the endurance training from the same sport in a concurrent training regime and visa versa?

(c) The effect that the training status of individuals has on recovery dynamics post-training

A common trend in the all three studies of this investigation was the influence that the training age and/or VO$_2$ max of the subjects had on both the recovery of force generating capacity and the physiological cost during subsequent exercise bouts. The majority of previous concurrent training studies have used untrained or physical active subjects that have not been involved in strength or endurance training. Therefore, further research comparing the responses of trained and untrained subjects in concurrent training programmes is needed to determine if the level of training adaptation of trained subjects is indeed an influencing factor in recovery dynamics post-exercise. The potential of well-trained endurance athletes to respond to strength training in light of their existing level of endurance development also requires further investigation.

(d) The time course of recovery between and following concurrent training sessions

In this series of studies, three hours rest between training sessions was found to be insufficient to enable complete recovery of force generating capacity and metabolic process prior to the commencement of a second training session. Therefore, further research of the time course of recovery following various intensities and durations of strength and endurance training are required to determine the rest intervals necessary between concurrent training sessions in order to avoid the possible negative residual effects of one training session on another and thereby maximise training adaptation.

(e) The possible relationship between muscle damage and concurrent training adaptations

In the present thesis, cycling exercise was found not to increase the level of CK activity following the two training sequences in Study 2 any more than that found for a single bout of high-intensity strength training. In addition, there is evidence of a possible relationship between the type of strength training and the mode of endurance training completed in
concurrent training studies and the impedance of strength training. In light of this, an investigation of the type (damage to contractile structures and/or neuromuscular disruptions) and the severity of muscle damage sustained following various combinations of concurrent training sessions needs to be completed using more direct methods such as electron microscopy. The cumulative effect of this muscle damage following repeated applications of the concurrent training sessions also needs to be examined.

(f) Examination of the cumulative effects of the acute and chronic mechanisms on strength and endurance adaptation

The present series of studies demonstrated that some of the proposed mechanisms for the impedance of strength and/or endurance development are affected by the sequence of concurrent training sessions. However, more extensive research is required both during and immediately following the respective training sequences as well as over successive applications of the training stimuli, to determine whether and how

- alterations in motor unit recruitment patterns influence the performance of concurrent training sessions;
- the continued reduction in muscle glycogen content interferes with performance of and recovery from concurrent training sessions performed on subsequent days;
- the accumulated responses of hormones associated with muscle growth and development such as testosterone, cortisol and IGF are altered compared to single mode training sessions;
- the responses of genes associated with muscle growth and metabolism are the same over successive applications of the training sequences and what is the cumulative effect compared to single training modes; and

the roles of these factors in the long-term development of strength and endurance.

(g) Overtraining threshold

Overtraining has been questioned as a possible source of the impedance of strength and endurance development in concurrent training regimes. Whilst the increased physiological stress of those training sessions completed second in the training sequences was clearly
evident in the current series of studies, it did not constitute overtraining. It did however raise the question does the cumulative effect of such stress reach a threshold that retards the level of adaptation? Furthermore, is the affect the same for both strength and endurance training? Therefore, the possibility of an overtraining threshold as suggested by Leveritt et al (1999) for each type of training (strength and endurance) requires investigation.

8.9. Concluding Remarks

The current series of studies showed that the chosen training variables (intensity, duration and mode of strength training) and the sequence in which concurrent training sessions are completed, did affect the rate of recovery post-training and the extent to which one training session affects another training session completed on the same day. Further, whilst changes in hormonal responses and skeletal muscle gene expression may be chronic mechanisms responsible for the reduced level of adaptation found following some concurrent training regimes, it appears that residual fatigue is the most notable mechanism contributing to the interference of concurrent training session performance. The residual fatigue from the prior training session increasing the physiological stress of subsequent training sessions in the training sequence. This in turn influencing the rate of recovery of force generating capacity, choice of substrate, level of energy expenditure, and the hormonal and gene expression responses during and post-exercise. This thesis has contributed to a greater understanding of how the completion of concurrent training sessions on the same day affects the performance of and recovery from the training sessions. However, further research is required to fully understand how the differences in the acute responses between the strength training protocols and training sequences may affect long-term strength and/or endurance development.
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APPENDICES

Appendix A: Pre-Participation Health Status Assessment Questionnaire

Appendix B: Study 1 – Information Sheet

Appendix C: Study 2 – Information Sheet

Appendix D: Study 3 – Information Sheet

Appendix E: Informed Consent for Exercise Testing

Appendix F: Statistical Tables

Appendix G: Test-Retest Reliability of the Discontinuous Incremental Cycling Efficiency Test
This form is used as a pre-participation health and risk factor screening device and should be completed prior to the commencement of an exercise test.

The information obtained in this medical assessment will be kept as CONFIDENTIAL. Only the staff member related to the exercise test may access the information.

Section (1) to (8) should be completed by the client before seeing a medical practitioner.

Section (9) and (10) should be completed by an exercise science professional or medical practitioner.

Client's Surname (Mr., Mrs., Ms.): ______________________________
Given Names: ________________________________________________
Date of Birth: ________________________________________________
Address: ____________________________________________________
________________________________________ Postcode: _________
Contact Telephone: ____________ (Home) _________________(Work)
Contact in case of an Emergency: Name: ______________________
Telephone (H): ________________
(W): _____________________
Relationship: ________________

Reasons for exercise testing:

_______________________________________________________________________
(1) FAMILY MEDICAL HISTORY

Has any near relative brother (B), sister (S), father (F), mother (M), grandparents (GP) suffered:

(Please tick the appropriate column)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No</th>
<th>Yes</th>
<th>Relation</th>
<th>Age</th>
<th>Remarks /Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoplexy (stroke)</td>
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<tr>
<td>Congenital heart trouble</td>
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<tr>
<td>Rheumatic heart disease</td>
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<td>Heart operation</td>
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<td>Angina</td>
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<tr>
<td>Heart attack</td>
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<tr>
<td>Sudden death</td>
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<td>High blood pressure</td>
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<tr>
<td>High cholesterol</td>
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<tr>
<td>'Hardening of arteries'</td>
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<tr>
<td>Asthma</td>
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<tr>
<td>Lung disorder</td>
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<tr>
<td>Bronchitis, emphysema</td>
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<tr>
<td>Hay fever</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Gout</td>
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<tr>
<td>Arthritis</td>
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<tr>
<td>Epilepsy</td>
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</tbody>
</table>
(2) PAST MEDICAL HISTORY

Have you suffered any of the following conditions at any time:

(Please tick the appropriate column)

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic or scarlet fever</td>
<td></td>
<td></td>
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<tr>
<td>Heart trouble or murmur</td>
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<tr>
<td>Heart palpitation</td>
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<tr>
<td>High blood pressure</td>
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<tr>
<td>Heart attack</td>
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<tr>
<td>Chest pain/Angina</td>
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<tr>
<td>Stroke</td>
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<tr>
<td>Disease of arteries or veins</td>
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<tr>
<td>Undue limiting shortness of breath with exercise</td>
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<tr>
<td>Fainting or blackout</td>
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<tr>
<td>Loss of consciousness or fainting with exercise</td>
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<tr>
<td>Epilepsy</td>
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<tr>
<td>Lung or bronchial disease</td>
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<tr>
<td>Asthma</td>
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<td></td>
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<tr>
<td>Hay fever</td>
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<td></td>
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<tr>
<td>Anaemia</td>
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<td></td>
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<tr>
<td>Diabetes</td>
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<td></td>
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<tr>
<td>Thyroid disease</td>
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<tr>
<td>Arthritis, rheumatism or gout spondylitis,</td>
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<tr>
<td>Disc trouble or back injury</td>
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<tr>
<td>Serious accident or injury</td>
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<tr>
<td>Surgical operation</td>
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<tr>
<td>Congenital abnormality</td>
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<tr>
<td>Other serious illness (or conditions that may affect exercise)</td>
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<tr>
<td>For female only:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Having normal/regular periods</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(3) SOCIAL HISTORY

Martial Status: _______________________ Number of Children _____________

Occupation: ____________________________

(4) HABITS

Please tick and fill in your usual habit of consumption of the following substances:

Alcoholic beverage:

( ) Nil

( ) Every day: number of drinks per day: _____________

( ) Irregular: number of drinks per week: _____________

Smoking:

( ) Never smoked

( ) Gave up _____ years ago

( ) Now smoke

Cigarettes: ___________ per day

Pipe: ___________ per day

Cigars: ___________ per day

(5) DIETARY SUPPLEMENTS

Please tick if you regularly take any of the following:

( ) Vitamins: Type: _________________________________

( ) Minerals: Type: _________________________________

( ) Iron: Type: _________________________________

( ) Other compounds: _________________________________
(6) PRESENT MEDICAL CONDITION

This section should be completed at the **INITIAL EXAMINATION** and also at examination **IMMEDIATELY PRIOR TO THE EXERCISE TEST**.

Are you currently suffering or have you in the recent past suffered any of the following conditions.

(Please tick the appropriate column)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Initial Exam</th>
<th>Second Exam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stuffy nose or sore throat</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tonsillitis, glandular fever</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Diarrhoea/vomiting</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Headaches</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pain in chest, left arm or neck at rest, or during physical activities</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Heart palpitations</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cramp in legs</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Abnormal loss of blood</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Indigestion or constipation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Swollen, stiff or painful joints</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Backache</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sports injury or other injury</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Any deterioration in training or competitive performance</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Any other conditions that may contraindicate to exercise or affect exercise capacity</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>For female only:</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Currently in pregnancy</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

If yes, provide details
(7) CURRENT MEDICATION

This section should also be checked **IMMEDIATELY PRIOR TO THE EXERCISE TEST.**

State the name and dosage of any drugs or medicines that you are taking regularly:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Time of last dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(8) EXERCISE HISTORY

(a) Present sport or exercise activity:

___________________________________________________________

Age at which dedicated sport participation: ____________________

Previous sports or exercise: ___________________________________

(b) Current training or exercise program (brief description):

<table>
<thead>
<tr>
<th>Type of activity</th>
<th>Hours per day</th>
<th>Days per week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) Personal best performance in sports competitions:

____________________________________________________________

____________________________________________________________

____________________________________________________________
(9) PHYSICAL EXAMINATION
This section should be completed by the medical practitioner.

(a) General appearance including glands and lymph nodes

(b) Cardiovascular system
(i) Peripheral vessel and pulses
(ii) Neck veins
(iii) Apex beat position
(iv) Heart sounds
(v) Resting heart rate
(vi) Blood pressure: Lying _________mmHg, Standing _________mmHg
(vii) 12 leads ECG (a copy of resting ECG should be on file).

(c) Respiratory system

(d) Abdomen

(e) Nervous system

(f) Fundi

(g) Locomotor system

(h) Varicose veins

(i) Urinalysis:
Protein: ___________ Sugar: ______________
Specific Gravity: _________ Other results: ___________

(j) Blood lipid profile:
TC: ___________ LDL: ______________
Triglyceride: ___________ Other results: ___________

(k) Other examinations:
(10) MEDICAL PRACTITIONER'S SUMMARY:

(a) Comments (detail any significant abnormalities or reservations):

________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________

(b) Recommendations:

The medical practitioner should underline and initial the appropriate clause:

(i) Fit to undergo maximal exercise tests.

(ii) Not fit to undergo maximal exercise test, but may undergo submaximal test without special precautions.

(iii) Not fit to undergo maximal exercise test but may undergo submaximal test with following precaution:

Precaution: _____________________________________________
________________________________________________________________________________________

(iv) Not fit to undergo any exercise test.

Signed: _____________________________ Name: _____________________________

Date: _____________________________

Contact Telephone Number: (Work) ___________________ (A/H) ______________
Appendix B

Study 1 – Information Sheet

You are invited to participate in a study titled “The effects of high and low intensity strength training and modes of strength training on cycling efficiency and blood parameters post training”. This study seeks to determine if varying intensity and mode of strength training changes cycling efficiency 3 hours and blood parameters 3 and 24 hours post training.

Participant Screening and Preparation

If you decide to participate, you will be required to complete a Pre-Participation Health Status Assessment Questionnaire in order to determine your current health status and suitability as a participant. In order to control for possible outside influences that may be impact upon your results during the test and training sessions, you are asked to,

1. Avoid participating in any leg strength training within 48 hours of a test.
2. Avoid participating in any strenuous exercise within 24 hours of a test.
3. Refrain from consuming any caffeine, supplements or drugs, which may affect your performance, within three hours of a test.
4. Refrain from consuming any alcohol within 24 hours of a test.
5. Refrain from consuming any food or drink (except water) within 2 hours of a test/training session.

Dietary Intake Diary

Because of the sensitive nature of some of the test variables to food and fluid intake, you will be required to maintain a dietary intake diary from the morning of the first testing day to after the day’s last test session. You will be asked to replicate this dietary intake on the remaining strength training days.

Participant Attendance

As a participant of this study you will be required to attend a familiarisation session followed by 5 testing/training sessions over a period of 4-5 weeks. The first two sessions will be control sessions (VO₂ max test and cycling efficiency test) followed in random order by single bouts of strength, strength endurance and hill cycling strength training over 3 weeks. These sessions will require you to attend the laboratory to provide a blood sample one-hour before undertaking an isometric strength/muscle activation test prior to each bout of strength training. Three hours after the completion of each strength training session you will again be required to complete an isometric strength/muscle activation test followed by an unloaded and loaded cycling efficiency test. Twenty-four hours after each strength training session you will be required to attend the laboratory to provide a blood sample. Each session will last approximately 90 minutes.
Familiarisation Session

You will be required to attend a familiarisation session in the week prior to commencing testing. During this session you will be familiarised with the gas collection equipment, cycle ergometer and the strength training equipment that will be used in the respective test and training sessions. During this session you will also be fully instructed on all the test procedures. The familiarisation session will also be used as the test time to determine your leg-press one repetition maximum (1 RM).

Test and Training Protocols

Throughout this study you will be required to undertake a number of tests and training protocols as outlined below.

*Isometric Strength Assessment*: involves performing a number of single leg-extension maximal voluntary isometric contraction trials.

*Muscle Activation Assessment (optional)*: involves applying a brief electrical stimulus to the quadriceps femoris muscle during a maximal voluntary isometric contraction and immediately after when the muscle is relaxed.

*Cycling VO₂ max Test*: involves maintaining a constant cadence whilst the workload is increased in small increments each minute until the designated workload can no longer be maintained.

*Cycling Efficiency Tests*: involves cycling for 5-10 minutes at four sub-maximal workloads with 2 minute rest intervals.

*Strength Training Protocols*: The strength and strength endurance training protocols consist of a six sets of your leg-press 6 and 20 RM, respectively. The hill cycling strength training consists of six 20 second repetitions.

*Blood Specimens*: A small specimen of capillary blood will be collected before and immediately post the strength training sessions, VO₂ max tests, cycling efficiency tests and at 24 hours post the strength training sessions.

*Body Temperature Monitoring*: Your body temperature will be measured pre, during and post the cycling efficiency tests.

Possible Risks and Discomforts

*Blood Specimen and Respiratory Gas Collection*: Standard handling and cleaning precautions (gloves, sterilised equipment etc) will be used during all blood specimen and respiratory gas collection to ensure that the risk of infection or transmission of diseases is minimised. All blood specimen collection will be carried out in the Exercise Science laboratory by qualified staff.

*Muscle Activation, Strength and Cycling Tests/Training*: All the tests and training that will be carried out in this study are a very stressful and as such may leave you with temporary local muscle soreness and/or a general feeling of fatigue.
Responsibilities of the Researcher

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

Responsibilities of the Participant

To ensure your safety throughout the study, if at any time you wish to discontinue a test due to feeling ill, pain or discomfort, please inform the supervising staff immediately. If at any time you are unsure of any of the test procedures, would like further information or have questions, please do not hesitate to ask or contact the supervising staff.

Freedom of Consent

If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time. However, we would appreciate you letting us know your decision.

Inquiries

If you have any questions, please do not hesitate to ask:

Dr Shi Zhou (Room P1.54, Phone 6620 3991), Dr Allan Davie (Room P1.55, Phone 6620 3236) or Mr Glen Deakin (Room P1.14, Phone 6620 3232), School of Exercise Science and Sport Management, Southern Cross University.
Appendix C

Study 2 – Information Sheet

You are invited to participate in a study titled “The effect of the sequence of strength and endurance training on cycling efficiency and blood parameters post training”. This study seeks to determine if the sequence of strength and endurance training, completed on the same day, changes cycling efficiency 3 hours and blood parameters 3 and 24 hours post training.

Participant Screening and Preparation

If you decide to participate, you will be required to complete a Pre-Participation Health Status Assessment Questionnaire in order to determine your current health status and suitability as a participant. In order to control for possible outside influences that may be impact upon your results during the test and training sessions, you are asked to,

- Avoid participating in any leg strength training within 48 hours of a test.
- Avoid participating in any strenuous exercise within 24 hours of a test.
- Refrain from consuming any caffeine, supplements or drugs, which may affect your performance, within three hours of a test.
- Refrain from consuming any alcohol within 24 hours of a test.
- Refrain from consuming any food or drink (except water) within 12 hours of the morning blood specimen and within 2 hours of a test/training session.

Dietary Intake Diary

Because of the sensitive nature of some of the test variables to food and fluid intake, you will be required to maintain a dietary intake diary from the morning of the first testing day to after the day’s last test session. You will be asked to replicate this dietary intake on the remaining training days.

Participant Attendance

As a participant of this study you will be required to attend a familiarisation session followed by 4 testing/training sessions over a period of 3-4 weeks. The first two sessions will be control sessions (VO2 max test and cycling efficiency test) followed by two different sequences of single day strength and cycling endurance training over 2 weeks. These sessions will require you to attend the laboratory to provide a blood sample one hour before undertake an isometric strength/muscle activation test prior to each day’s initial training session. Three hours after the completion of the first training sessions you will be required to complete another training session. This will be followed three hours later by an isometric strength/muscle activation test and cycling efficiency test. Twenty-four hours after the initial training session you will be required to attend the laboratory to provide a blood sample. Each session will last approximately 90 minutes.
Familiarisation Session

You will be required to attend a familiarisation session in the week prior to commencing testing. During this session you will be familiarised with the gas collection equipment, cycle ergometer and the strength training equipment that will be used in the respective test and training sessions. During this session you will also be fully instructed on all the test procedures. The familiarisation session will also be used as the test time to determine your leg-press, bench-press and lat pull down one or six repetition maximum.

Test and Training Protocols

Throughout this study you will be required to undertake a number of tests and training protocols as outlined below.

*Isometric Strength Assessment*: involves performing a number of single leg-extension maximal voluntary isometric contraction trials.

*Muscle Activation Assessment (optional)*: involves applying a brief electrical stimulus to the quadriceps femoris muscle during a maximal voluntary isometric contraction and immediately after when the muscle is relaxed.

*Cycling VO2 max Test*: involves maintaining a constant cadence whilst the workload is increased in small increments each minute until the designated workload can no longer be maintained.

*Cycling Efficiency Tests*: involves cycling for 5-10 minutes at four sub-maximal workloads with 2-minute rest intervals.

*Training Protocols*: The strength training protocol will consist of six or four sets of your leg-press, bench-press and lat pull down 6 RM. The cycling endurance training will consist of 60 minutes of cycling at a workload corresponding to 60% of your VO2 max.

Blood Specimens: A small specimen of capillary and/or venous blood will be collected before and immediately post the strength training sessions, VO2 max tests, cycling efficiency tests and at 24 hours post the strength training sessions.

*Body Temperature Monitoring*: Your body temperature will be measured pre, during and post the cycling efficiency tests.

Possible Risks and Discomforts

*Blood Specimen and Respiratory Gas Collection*: Standard handling and cleaning precautions (gloves, sterilised equipment etc) will be used during all blood specimen and respiratory gas collection to ensure that the risk of infection or transmission of diseases is minimised. All blood specimen collection will be carried out in the Exercise Science laboratory by qualified staff.

*Muscle Activation, Strength and Cycling Tests/Training*: All the tests and training that will be carried out in this study are a very stressful and as such may leave you with temporary local muscle soreness and/or a general feeling of fatigue.
Responsibilities of the Researcher

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

Responsibilities of the Participant

To ensure your safety throughout the study, if at any time you wish to discontinue a test due to feeling ill, pain or discomfort, please inform the supervising staff immediately. If at any time you are unsure of any of the test procedures, would like further information or have questions, please do not hesitate to ask or contact the supervising staff.

Freedom of Consent

If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time. However, we would appreciate you letting us know your decision.

Inquiries

If you have any questions, please do not hesitate to ask:

Dr Shi Zhou (Room P1.54, Phone 6620 3991), Dr Allan Davie (Room P1.55, Phone 6620 3236) or Mr Glen Deakin (Room P1.14, Phone 6620 3232), School of Exercise Science and Sport Management, Southern Cross University.
Appendix D

Study 3 – Information Sheet

You are invited to participate in a study examining “the effect of the sequence of strength and endurance training on the response of hormones and gene expression post training”. This study seeks to determine if the sequence of strength and endurance training, completed on the same day, changes the level of response of these variables over the first 24 hours post training.

Participant Preparation

In order to control for possible outside influences that may be impact upon your results during the test and training sessions, you are asked to,

- Avoid participating in any leg strength training within 48 hours of a test.
- Avoid participating in any strenuous exercise within 24 hours of a test.
- Refrain from consuming any caffeine, supplements or drugs, which may affect your performance, within three hours of a test
- Refrain from consuming any alcohol within 24 hours of a test.
- Refrain from consuming any food or drink (except water) within 12 hours of the morning blood specimen (pre training session).

Dietary Intake

Because of the sensitive nature of some of the test variables to food and fluid intake, your dietary intake for each training day will be predetermined and provided. The same dietary intake will be replicated across all training days.

Participant Attendance

As a participant of this study you will be required to attend a familiarisation session followed by 5 testing/training sessions over a period of 5-6 weeks. The first three sessions will be control sessions (VO₂ max test and a single strength and endurance training session) followed by two different sequences of single day strength and endurance cycling training. These sessions will require you to attend the laboratory to provide a muscle and/or blood specimen prior to each day’s initial training session. An hour later you will complete either an hour strength training session or three hour endurance cycling session. Three hours after the completion of the first training session you will be required to undertake the second training session (excluding the control days). Blood specimens will be taken prior to, immediately following and every 15 minutes for the first hour post training and each hour after that for a period of 3 hours. Twenty-four hours after the initial and last blood samples on the first day you will be required to attend the laboratory to provide another blood specimen. Muscle biopsies will be taken prior to and following each training session as well as 3 hours post the final training session. Twenty-four hours after the biopsy following the strength training session you will be required to attend the laboratory for another muscle biopsy.
Familiarisation Session

You will be required to attend a familiarisation session in the week prior to commencing testing. During this session you will be familiarised with the gas collection equipment, cycle ergometer and the strength training equipment that will be used in the respective test and training sessions. During this session you will also be fully instructed on all the test procedures. The familiarisation session will also be used as the test time to determine your leg-press one repetition maximum (1 RM) and leg-extension/curl 6 RM.

Test and Training Protocols

Throughout this study you will be required to undertake a number of tests and training protocols as outlined below.

Cycling VO₂ max Test: involves maintaining a constant cadence whilst the workload is increased in small increments each minute until the designated workload can no longer be maintained.

Training Protocols: The strength training session will consist of six sets of your leg-press and four sets of your leg-extension and leg-curl 6 RM. The cycling endurance training will consist of 3 hours of continuous cycling at a workload corresponding to 60% of your VO₂ max.

Possible Risks and Discomforts

There are possible risks and discomforts that may occur during this study including;

- Temporary discomfort from muscle biopsy or blood sampling techniques,
- Muscle/tendon injuries,
- Temporary muscle soreness and/or joint pain,
- General and/or local muscle fatigue.

The chance of such incidents is minimal, however, precautions will be taken to further reduce the risk of these irregularities occurring, including;

- The completion of a warm-up prior to each testing/training session.
- The use mechanical stops and spotters to limit your range of motion and provide assistance in the event of a failed lift during the strength testing/training sessions.
- Monitoring of your physical condition during each testing session by qualified exercise science staff.
- Standard handling and cleaning precautions will be used during all blood and muscle specimen and respiratory gas collection to ensure that the risk of infection or transmission of diseases is minimised. All specimen collection will be carried out in the Exercise Science laboratory by qualified staff.

Responsibilities of the Participant

To ensure your safety throughout the study, if at any time you wish to discontinue a test due to feeling ill, pain or discomfort, please inform the supervising staff immediately. If at any time you are unsure of any of the test procedures, would like further information or have questions, please do not hesitate to ask.
Inquiries

Glen Deakin  (Telephone: 35 3216 81)
Room 428, August Krogh Institute
University of Copenhagen
E-mail: GBDeakin@aki.ku.dk
Appendix E

Informed Consent for Exercise Testing

School of Exercise Science & Sport Management
Southern Cross University

I, ______________________________ of __________________________________
(name) (residential address)

hereby consent to voluntarily engage in the exercise test entitled _______________________, as
described in the attached information sheet(s).

Before I undergo the test, if necessary, I will be examined by a Medical Practitioner or a qualified
Exercise Science Professional to determine if I have any condition that would indicate that I should
not engage in the test. Qualified personnel will observe my physical condition directly throughout the
test. If abnormal signs appear, the test will be stopped. I may stop at any time during the test because
of feelings of fatigue or any other discomfort.

I recognise that despite the above precautions there exists the possibility of certain changes and/or
risks occurring during the test. These may include caffeine, supplement or drug withdrawal, abnormal
blood pressure responses, fainting, muscle/tendon/bone injuries, irregularities of heartbeat, and/or very
rare instances of heart attack. Every effort will be made to minimise the risks by the preliminary
examination and by observations during testing. I will report unusual feelings during testing to testing
staff. Emergency procedures and trained personnel are available to deal with unusual situations should
they arise.

I have read, prior to signing this form, the attached information sheet(s) that explains the purpose,
procedures and possible discomforts and risks involved in the prescribed tests. I have also been given a
full verbal explanation by the testing personnel and opportunities to ask questions in regard to the test.
I will ask for further explanations should any concerns or questions arise.

I authorise the details of my performance assessment and personal particulars to be included
in statistical summaries for the purpose of research and advanced education, apart from which
the information that may reveal my individual identity will remain confidential within the
testing personnel in the School.

Signature of the Subject_________________________________ Date ____________
Signature of a witness   _________________________________ Date  ___________
(Who shall be independent of the test)
Signature of the testing personnel _________________________ Date __________

Any complaints or queries regarding this project that cannot be answered by the person responsible for
this research should be forwarded to:
Mr John Russell, Graduate Research College, Southern Cross University.
Ph: (02) 6620 3705    Fax: (02) 6622 3180   Email: jrussell@scu.edu.au
Appendix F

Statistical Tables

F. 1. Effect of Test Order
Results of the statistical analysis for the between-subjects factor (test order) for Studies 1, 2 and 3 are outlined below. The results have been summarised and indicate the variable measured, F-ratio (F), degrees of freedom (df) and the corresponding level of significance (Sig.). For those analyses that had multiple comparisons (e.g. Study 1A – efficiency test variables), the statistical values for each comparison are indicated under the respective titled column (1 and 2 or A, B and C or CW and WC).

F.2. STUDY 1 – Experiment 1A

Table F.2.1. Comparison of the strength training variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL Concentration</td>
<td>0.321</td>
<td>2, 6</td>
<td>.737</td>
</tr>
<tr>
<td>CK activity</td>
<td>2.285</td>
<td>2, 4</td>
<td>.218</td>
</tr>
</tbody>
</table>

Table F.2.2. Comparison of the maximal voluntary contraction and muscle activation responses pre and post the strength training protocols.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Torque</td>
<td>0.549</td>
<td>2, 6</td>
<td>.604</td>
</tr>
<tr>
<td>Mean Torque</td>
<td>0.590</td>
<td>2, 6</td>
<td>.583</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak MA</td>
<td>1.865</td>
<td>2, 6</td>
<td>.234</td>
</tr>
<tr>
<td>Mean MA</td>
<td>1.058</td>
<td>2, 6</td>
<td>.404</td>
</tr>
</tbody>
</table>
Table F.2.3. Comparison of the efficiency test variables post the strength training protocols.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>0.205</td>
<td>0.430</td>
<td>2, 6</td>
</tr>
<tr>
<td>RR</td>
<td>0.791</td>
<td>0.193</td>
<td>2, 6</td>
</tr>
<tr>
<td>( V_E )</td>
<td>0.473</td>
<td>0.457</td>
<td>2, 6</td>
</tr>
<tr>
<td>( V_O_2 )</td>
<td>0.603</td>
<td>0.151</td>
<td>2, 6</td>
</tr>
<tr>
<td>GE</td>
<td>0.359</td>
<td>0.438</td>
<td>2, 6</td>
</tr>
<tr>
<td>NE</td>
<td>0.438</td>
<td>0.438</td>
<td>2, 6</td>
</tr>
<tr>
<td>BL Concentration.</td>
<td>0.007</td>
<td>0.156</td>
<td>2, 4</td>
</tr>
<tr>
<td>TT</td>
<td>4.516</td>
<td>0.984</td>
<td>2, 5</td>
</tr>
<tr>
<td>GBM</td>
<td>1.058</td>
<td>1.058</td>
<td>2, 6</td>
</tr>
<tr>
<td>RPE</td>
<td>0.866</td>
<td>0.866</td>
<td>2, 6</td>
</tr>
</tbody>
</table>

F.3. STUDY 2

Table F.3.1. Comparison of the weight and endurance cycle training variables.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight Training</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL Concentration</td>
<td>0.847</td>
<td>1, 6</td>
<td>.393</td>
</tr>
<tr>
<td>CK activity</td>
<td>1.303</td>
<td>1, 6</td>
<td>.297</td>
</tr>
<tr>
<td><strong>Endurance Cycle Training</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>3.883</td>
<td>1, 6</td>
<td>.096</td>
</tr>
<tr>
<td>RR</td>
<td>0.066</td>
<td>1, 6</td>
<td>.807</td>
</tr>
<tr>
<td>( V_E )</td>
<td>0.002</td>
<td>1, 6</td>
<td>.966</td>
</tr>
<tr>
<td>( V_O_2 )</td>
<td>0.240</td>
<td>1, 6</td>
<td>.641</td>
</tr>
<tr>
<td>RER</td>
<td>0.641</td>
<td>1, 6</td>
<td>.454</td>
</tr>
<tr>
<td>GE</td>
<td>0.248</td>
<td>1, 6</td>
<td>.636</td>
</tr>
<tr>
<td>NE</td>
<td>0.422</td>
<td>1, 6</td>
<td>.540</td>
</tr>
<tr>
<td>BL Concentration</td>
<td>1.659</td>
<td>1, 6</td>
<td>.245</td>
</tr>
<tr>
<td>TT</td>
<td>0.437</td>
<td>1, 6</td>
<td>.533</td>
</tr>
<tr>
<td>GBM</td>
<td>0.760</td>
<td>1, 6</td>
<td>.417</td>
</tr>
<tr>
<td>RPE</td>
<td>0.432</td>
<td>1, 6</td>
<td>.536</td>
</tr>
</tbody>
</table>
Table F.3.2. Comparison of the maximal voluntary contraction and muscle activation responses for the control day and sequence days.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MVC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Torque</td>
<td>1.841</td>
<td>1, 6</td>
<td>.224</td>
</tr>
<tr>
<td>Mean Torque</td>
<td>1.920</td>
<td>1, 6</td>
<td>.215</td>
</tr>
<tr>
<td><strong>MA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak MA</td>
<td>3.619</td>
<td>1, 6</td>
<td>.106</td>
</tr>
<tr>
<td>Mean MA</td>
<td>2.425</td>
<td>1, 6</td>
<td>.170</td>
</tr>
</tbody>
</table>

Table F.3.3. Comparison of the hormone responses for the control day and sequence days.

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>0.056</td>
<td>1, 3</td>
<td>.828</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.624</td>
<td>1, 3</td>
<td>.484</td>
</tr>
<tr>
<td>Testosterone/Cortisol Ratio</td>
<td>0.129</td>
<td>1, 3</td>
<td>.743</td>
</tr>
</tbody>
</table>

Table F.3.4. Comparison of the efficiency test variables for the control day and sequence days.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison</strong></td>
<td>(1) (2)</td>
<td>(1) (2)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>0.423</td>
<td>1, 6</td>
<td>.540 .261</td>
</tr>
<tr>
<td>RR</td>
<td>5.084</td>
<td>1, 6</td>
<td>.065 .628</td>
</tr>
<tr>
<td>VE</td>
<td>0.962</td>
<td>1, 6</td>
<td>.365 .935</td>
</tr>
<tr>
<td>VO₂</td>
<td>0.143</td>
<td>1, 6</td>
<td>.718 .647</td>
</tr>
<tr>
<td>GE</td>
<td>0.546</td>
<td>1, 6</td>
<td>.488</td>
</tr>
<tr>
<td>NE</td>
<td>0.013</td>
<td>1, 6</td>
<td>.915</td>
</tr>
<tr>
<td>BL Concentration</td>
<td>5.104</td>
<td>1, 3</td>
<td>.109 .873</td>
</tr>
<tr>
<td>TT</td>
<td>1.646</td>
<td>1, 5</td>
<td>.256 .089</td>
</tr>
<tr>
<td>GBM</td>
<td>0.699</td>
<td>1, 6</td>
<td>.435</td>
</tr>
<tr>
<td>RPE</td>
<td>5.377</td>
<td>1, 6</td>
<td>.060</td>
</tr>
</tbody>
</table>
**F.4. STUDY 3**

**Table F.4.1.** Comparison of the weight training variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL Concentration</td>
<td>2.150</td>
<td>1, 6</td>
<td>.193</td>
</tr>
<tr>
<td>HR</td>
<td>0.858</td>
<td>1, 6</td>
<td>.390</td>
</tr>
<tr>
<td>Peak</td>
<td>0.711</td>
<td>1, 6</td>
<td>.431</td>
</tr>
</tbody>
</table>

**Table F.4.2.** Comparison of the endurance cycle training variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL Concentration</td>
<td>1.749</td>
<td>1, 6</td>
<td>.234</td>
</tr>
<tr>
<td>HR</td>
<td>0.002</td>
<td>1, 4</td>
<td>.971</td>
</tr>
<tr>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td>1.179</td>
<td>1, 4</td>
<td>.339</td>
</tr>
<tr>
<td>RPE</td>
<td>0.344</td>
<td>1, 5</td>
<td>.583</td>
</tr>
<tr>
<td>RR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>{ No Statistics }</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table F.4.3.** Muscle glycogen content.

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle Glycogen Content</td>
<td>1.243</td>
<td>1, 4</td>
<td>.327</td>
</tr>
</tbody>
</table>
Table F.4.4. Hormones – Testosterone, cortisol and testosterone/cortisol ratio.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F (WC)</th>
<th>df</th>
<th>Sig. (WC)</th>
<th>(CW)</th>
<th>df</th>
<th>Sig. (CW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testosterone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C vs CW</td>
<td>0.779</td>
<td>1,4</td>
<td>.427</td>
<td>.309</td>
<td>.622</td>
<td></td>
</tr>
<tr>
<td>W vs WC</td>
<td>0.667</td>
<td>1,4</td>
<td>.460</td>
<td>.379</td>
<td>.515</td>
<td></td>
</tr>
<tr>
<td>C vs W</td>
<td>0.697</td>
<td>1,4</td>
<td>.451</td>
<td>.495</td>
<td>.564</td>
<td></td>
</tr>
<tr>
<td>CW vs WC</td>
<td>0.589</td>
<td>1,4</td>
<td>.486</td>
<td>.171</td>
<td>.556</td>
<td></td>
</tr>
<tr>
<td><strong>Cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C vs CW</td>
<td>0.001</td>
<td>1,4</td>
<td>.974</td>
<td>.628</td>
<td>.646</td>
<td></td>
</tr>
<tr>
<td>W vs WC</td>
<td>0.260</td>
<td>1,4</td>
<td>.637</td>
<td>.355</td>
<td>.650</td>
<td></td>
</tr>
<tr>
<td>C vs W</td>
<td>0.004</td>
<td>1,4</td>
<td>.952</td>
<td>.781</td>
<td>.513</td>
<td></td>
</tr>
<tr>
<td>CW vs WC</td>
<td>0.444</td>
<td>1,4</td>
<td>.542</td>
<td>.254</td>
<td>.519</td>
<td></td>
</tr>
<tr>
<td><strong>T/C Ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C vs CW</td>
<td>1.284</td>
<td>1,4</td>
<td>.321</td>
<td>.886</td>
<td>.318</td>
<td></td>
</tr>
<tr>
<td>W vs WC</td>
<td>0.317</td>
<td>1,4</td>
<td>.603</td>
<td>.356</td>
<td>.680</td>
<td></td>
</tr>
<tr>
<td>C vs W</td>
<td>0.873</td>
<td>1,4</td>
<td>.403</td>
<td>.624</td>
<td>.421</td>
<td></td>
</tr>
<tr>
<td>CW vs WC</td>
<td>0.007</td>
<td>1,4</td>
<td>.937</td>
<td>.220</td>
<td>.629</td>
<td></td>
</tr>
</tbody>
</table>

Table F.4.5. Gene Expression.

<table>
<thead>
<tr>
<th>Measure</th>
<th>F (WC)</th>
<th>df</th>
<th>Sig. (WC)</th>
<th>(CW)</th>
<th>df</th>
<th>Sig. (CW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myogenin</td>
<td>2.150</td>
<td>1,5</td>
<td>.202</td>
<td>.778</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MyoD</td>
<td>0.008</td>
<td>1,5</td>
<td>.933</td>
<td>.891</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHCI</td>
<td>2.385</td>
<td>1,5</td>
<td>.183</td>
<td>.296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHCIIa</td>
<td>1.704</td>
<td>1,5</td>
<td>.249</td>
<td>.760</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHCIIx</td>
<td>No Statistic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF1-2</td>
<td>3.627</td>
<td>1,5</td>
<td>.115</td>
<td>.240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDK4</td>
<td>2.480</td>
<td>1,5</td>
<td>.176</td>
<td>.254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGC1</td>
<td>0.206</td>
<td>1,5</td>
<td>.770</td>
<td>.081</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HKII</td>
<td>0.320</td>
<td>1,5</td>
<td>.596</td>
<td>.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>0.259</td>
<td>1,5</td>
<td>.633</td>
<td>.387</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS</td>
<td>1.224</td>
<td>1,5</td>
<td>.319</td>
<td>.445</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP3</td>
<td>1.604</td>
<td>1,5</td>
<td>.261</td>
<td>.941</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH-M</td>
<td>0.233</td>
<td>1,5</td>
<td>.650</td>
<td>.991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH-H</td>
<td>0.966</td>
<td>1,5</td>
<td>.371</td>
<td>.884</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPT1</td>
<td>2.885</td>
<td>1,5</td>
<td>.150</td>
<td>.661</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix G

Test-Retest Reliability of the Discontinuous Incremental Cycling Efficiency Test

G.1. Introduction
Reliability is a fundamental criteria used to judge the quality of a test (Thomas & Nelson, 1996). The significance of attaining reliable information is that reliability forms an integral part of validity, as a test that is not reliable cannot be valid (Baumgartner, 1989; Rothstein, 1985). In addition, athlete performance tests need to display high levels of precision in order to be able to detect the smallest changes in performance (Schabort, Hawley, Hopkins, Mujika, & Noakes, 1998). In view of the importance of having a reliable test, it was necessary to determine the test-retest reliability of the newly developed discontinuous incremental cycling efficiency test to ensure that it could provide a repeatable standardised test of the subjects’ cycling physiological status following the various training protocols in Studies 1 and 2.

G.2. Project Design
In order to determine the test-retest reliability of the newly developed discontinuous incremental cycling efficiency test, two identical tests were carried out in the weeks prior to the commencement of Study 1. After completing a familiarisation session, each subject completed a VO\textsubscript{2} max test, followed by two discontinuous incremental cycling efficiency tests (CE1 and CE2), on the same day of the week, over a period of two weeks. There was a minimum of three days between the VO\textsubscript{2} max test and the first cycling efficiency test in order to eliminate any possible carry over effects from one test to another. The cycling efficiency tests were also completed at the same time of the day across the study, to minimise the potential effects of biological rhythms (Reilly et al., 1997).

G.3. Subjects
Ten (10) trained male cyclists, consisting of seven cyclists who were actively competing in local road cycling or triathlon races and three recreational cyclists, volunteered for the study. Their mean (±SD) age, height, body mass and VO\textsubscript{2} max (absolute and relative) are outlined in Table G.1.
Table G.1. Descriptive characteristics of the ten subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.9 ± 4.6</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.79 ± 0.06</td>
</tr>
<tr>
<td>Body Mass (kilograms)</td>
<td>73.80 ± 5.83</td>
</tr>
<tr>
<td>VO2 max</td>
<td></td>
</tr>
<tr>
<td>Absolute (L.min⁻¹)</td>
<td>4.10 ± 0.60</td>
</tr>
<tr>
<td>Relative (mL.kg⁻¹.min⁻¹)</td>
<td>55.87 ± 6.71</td>
</tr>
</tbody>
</table>

The volunteers were selected for participation if they meet the following criteria.

- Aged between 18 and 40
- A minimum of 12 months cycling experience
- Currently cycling a minimum of 2-3 times per week with an approximate weekly duration total of 2-3 hours
- No contraindications to the testing procedure

The same Subject Preparation and Familiarisation, excluding the electrical stimulation and weight training equipment familiarisation, was utilised as described in the General Methodology and Materials Chapter for Studies 1 and 2 (Section 3.2). The Southern Cross University Human Research Ethics Committee approved the experimental procedure (ECN-01-37).

G.4. Methods, Instrumentation and Materials

The Methods, Instrumentation and Materials used in the reliability experiment were the same as those used in Studies 1 and 2 except for the following variations.

G.4.1. Laboratory Facilities

All tests were carried out under controlled environmental conditions (Table G.2), as described for Studies 1 and 2.
**Table G.2.** Room temperature, barometric pressure and relative humidity of the VO₂ max testing session and the two cycling efficiency test trials, CE1 and CE2 (Mean, brackets represent SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>VO₂ max</th>
<th>CE1</th>
<th>CE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature (°C)</td>
<td>22.6</td>
<td>22.7</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td>(0.9)</td>
<td>(0.6)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Barometric Pressure (mmHg)</td>
<td>760</td>
<td>761</td>
<td>761</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>48.6</td>
<td>46.1</td>
<td>46.5</td>
</tr>
<tr>
<td></td>
<td>(7.9)</td>
<td>(7.7)</td>
<td>(4.8)</td>
</tr>
</tbody>
</table>

**G.4.2. Statistical Analysis**

Descriptive statistics (mean and SD) were calculated for all measured variables.

The degree of test-retest reliability of the discontinuous incremental cycling efficiency test was determined by an Intraclass Correlation Coefficient (ICC), whilst the level of precision was determined by the calculation of the Technical Error of Measurement (TEM, absolute and relative) for each variable measured.

The ICC was calculated from the results of an analysis of variance (ANOVA). The mean squares (MS) from the ANOVA were combined in a ratio formula to give an ICC.

\[
\text{ICC} = \frac{\text{Between subjects MS} - \text{Within subjects MS}}{\text{Between subjects MS} + (k-1) \text{Within subjects MS}}
\]

Where, \(k\) = the number of measurements per subject (equal number of cases).

The relative TEM was calculated as:

\[
\text{TEM} = \sqrt{\frac{\Sigma d_i^2}{2n}}
\]

Where, \(d_i^2\) was the squared difference between the two measures of the \(ith\) subject and \(n\), the number of subjects.
A repeated measures ANOVA was also completed to determine if there was any significant difference between the dependent variables measured during the efficiency tests. An alpha level of .05 was used to indicate a level of significant difference for all ANOVA analysis. All statistical analysis was completed using the Statistical Package for Social Sciences (SPSS version 11.0 for windows, SPSS Inc.).

G.5. Results
The descriptive statistics for oxygen uptake (VO₂), heart rate (HR), workload (WL), ventilation (VE), respiratory rate (RR), blood lactate (BL) concentration, tympanic temperature (TT), gross efficiency (GE), net efficiency (NE) and ratings of perceived exertion (RPE) obtained from CE1 and CE2, are presented in Table G.3. The reliability statistics, Intraclass Correlation Coefficient (ICC) and Technical Error of Measurement (TEM, absolute and relative), for each test variable are also presented in Table G.3.

Ten of the twelve variables showed ICC values above .81 with GE, BL concentration and WL showing the highest ICCs with .99, .94 and .94, respectively. Seven of the ten variables measured showed relative TEM values below 5% with TT, WL and GE showing the lowest relative TEM values with 0.61%, 1.10% and 1.91%, respectively. These results indicated high levels of reliability and precision in testing.

A repeated measures ANOVA comparing VO₂, HR, WL, RR, BL, GE, NE, TT and the subjective response RPE revealed no significant difference between CE1 and CE2 (p>.05). The only variable that showed a significant difference was VE (p=.001).

G.6. Discussion
The results indicated that the discontinuous incremental cycling efficiency test using a five-minute period of unloaded cycling and three ten-minute workload periods at 20, 40 and 60% of VO₂ max, provided high reproducible measures of GE, cardiorespiratory responses, and associated physiological parameters such as BL concentration.

Gross efficiency, VO₂, RR and BL concentration along with WL exhibited high ICCs (≥.93), which indicated a high degree of reliability and that a high level of agreement would be
expected between successive measurements of these variables (Norton & Olds, 1996). Even though there is no universal criterion for interpreting reliability using the ICC method other than the closer the coefficient is to 1.00 the less error variance present (Norton & Olds, 1996), Vincent (1995) suggests a specific criterion for judging a measure’s reliability. He indicates that reliability coefficients above .90 are considered to reflect high reliability, from .80 to .89 moderate reliability and below .80, questionable reliability for physiological data. Using this criterion the above listed variables still indicate a high degree of reliability, whilst HR, NE and TT indicate a moderate level of reliability. The remaining variables VE and RPE would be considered as having questionable reliability and therefore, should be used with caution.

The reliability coefficient from this study for VO₂ (r=0.93) was consistent with reliability coefficients reported in the literature (.95) with respect to other sub-maximal cycling ergometer (Becque, Katch, Marks, & Dyer, 1993) and running protocols (Morgan, Martin, Krahenbuhl, & Baldini, 1991). Unfortunately, comparison to other cycle ergometer reliability investigations using sub-maximal protocols is limited because few investigations (Becque et al., 1993; Washburn & Montoye, 1985) have reported the reliability of other variables other than VO₂ even though other variables such as HR, GE, NE, WL and BL concentration were measured during the tests. Further, a variety of sub-maximal protocols have been utilised as too a variety of subjects and methods of calculation of reliability, which all influence the degree of reliability of physiological variables (Atkinson & Nevill, 1998). However, comparing the ICC for HR from this study to a similar designed protocol by Becque, Katch, Marks and Dyer (1993) who assessed the reliability of HR in addition to VO₂ during three 10-minute intervals at varying sub-maximal workloads during cycle ergometry using trained subjects, the ICC was within a similar range (.86-.89).

Apart from VE, which is discussed later in this discussion, RPE was the only other variable to show low test-retest reliability, with an ICC of .71. Reduced reliability in the range of .72-.82 for this variable is not uncommon and has been reported in previous studies (Lamb, Eston, & Corns, 1999; Mercer, 2001). This finding may be due to nature of the RPE, in that they come from the perceptions of effort by the subjects from both central and peripheral sources (Cafarelli, 1977; Ekblom & Goldberg, 1971; Pandolf & Noble, 1973) and as such may be influenced by the physiological and psychological stress of the subjects at the time of testing. Whilst precautions were taken to limit the influence of the two main sources of measurement error, biological variation and technical error (Becque et al., 1993; Katch, Sady, & Freedson,
by having all tests carried out under controlled environmental conditions, providing adequate rest days between all testing sessions, completing the efficiency tests at the same time of the day and on the same day of the week and the completion of familiarisation sessions prior to testing, as outlined in the General Methodology and Materials Chapter. It is possible that because the testing period for each subject was over a minimum of three weeks, that the subjects experienced some physiological or psychological changes (either positive or negative) as a result of their prior training practices or work/family life, which may have impacted upon their results, even though the subjects were asked not to alter their weekly training regimes for the duration of the testing period. Closer monitoring of training and arousal/stress levels prior to and between testing sessions may limit the influence of such changes in future research.

The literature does not indicate a criterion for determining what constitutes an acceptable level of measurement, however the lower the value for absolute and relative TEM the higher the level of precision, as high precision corresponds to low variability in successive measurements (Norton & Olds, 1996). The low absolute and relative TEM (< 4%) values for seven of the ten variables measured during the efficiency test including GE, NE, VO₂, HR, WL, RR and TT indicate high levels of precision. Whilst different formulas have been used to calculate precision levels, which make comparisons difficult, the results compare favourably with previous findings using sub-maximal exercise protocols (Armstrong & Costill, 1985; Becque et al., 1993; Moseley & Jeukendrup, 2001).

Three variables recorded relative TEM values greater than 5%, BL concentration (7.20%), RPE (6.93%) and VE (11.30%). The high TEM value for BL concentration appears to reflect low levels measured during the test rather than larger variations from test to test, as indicated by the same mean and SD for CE1 and CE2. The absolute TEM for BL concentration was 0.1 which indicates low variability however because the subjects were exercising at low intensities for the majority of the test, as reflected by the low mean value of 1.4 mmol.L⁻¹ recorded for each test, the absolute value represents a greater proportion of the total than if the exercise intensity was higher and a greater mean value was recorded. A similar scenario may also account for part of the high relative TEM value for RPE, in addition to the possible influence of measurement error as outlined above.
Minute ventilation was the only variable that recorded a relative TEM value higher than 10%. However, a large proportion of the variation in the absolute and relative TEM values for $V_E$ can be explained by a large variation in the test scores of two subjects. The susceptibility to variations in test scores from individual subjects is because the statistics are calculated from the squared difference of each subjects test scores (Baumgartner, 1989). Therefore, any noticeable differences are magnified by this method of calculation. Because the two subjects recorded greater variability in their test scores than the other subjects, their scores resulted in high absolute and relative TEM values being observed. The statistical analysis confirms the above observation, for if the abovementioned subjects were removed from the respective calculations there is a notable reduction in the relative and absolute TEM values to a higher level of precision. Further, the ICC would have increased to .71 indicating a higher degree of reliability than the .16 originally calculated. Whilst the two subject’s values were notably different from the rest of the subjects, they were not classified as extreme values. The revision of the ICC for $V_E$ demonstrates that the ICC is influenced by the range of measurements found for a variable within a group of subjects (Atkinson & Nevill, 1998). Therefore, consideration must be given to the influence of such variations in the evaluating of the reliability of a test. The finding of no significant difference between CE1 and CE2 for any of the test variables other than $V_E$, over the duration of the tests, indicates a metabolic similarity between the tests at a sub-maximal level. This finding adds support to the high degree of reliability of the efficiency test protocol.

In conclusion, the results showed that the newly developed discontinuous incremental cycling efficiency test using a five-minute unloaded cycling period and three ten-minute workload periods at 20, 40 and 60% of $VO_2$ max was a reliable method of determining sub-maximal cycling performance in trained cyclists. The present findings also indicated that the cycling efficiency test is sensitive enough to detect small changes in the majority of physiological responses of trained cyclists via high levels of precision.

G.7. References
Refer main reference list.
Table G.3. ICC and TEM (absolute and relative) values for VO<sub>2</sub>, HR, WL, V<sub>E</sub>, RR, BL, GE, NE, TT and RPE obtained from CE1 and CE2. The CE1 and CE2 values represent the mean and SD values over the duration of the cycling efficiency test.

<table>
<thead>
<tr>
<th>Test Variable</th>
<th>CE1</th>
<th>(± SD)</th>
<th>CE2</th>
<th>(± SD)</th>
<th>TEM</th>
<th>%TEM</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; (L.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.34</td>
<td>(0.11)</td>
<td>1.35</td>
<td>(0.12)</td>
<td>0.04</td>
<td>2.70</td>
<td>.93</td>
</tr>
<tr>
<td>Heart Rate (b.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>101.3</td>
<td>(10.8)</td>
<td>99.7</td>
<td>(9.1)</td>
<td>3.7</td>
<td>3.70</td>
<td>.86</td>
</tr>
<tr>
<td>Workload (watts)</td>
<td>119.5</td>
<td>(17.8)</td>
<td>119.5</td>
<td>(17.6)</td>
<td>1.3</td>
<td>1.10</td>
<td>.99</td>
</tr>
<tr>
<td>Ventilation (L.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>28.4</td>
<td>(2.3)</td>
<td>32.9</td>
<td>(3.6)</td>
<td>3.5</td>
<td>11.30</td>
<td>.16</td>
</tr>
<tr>
<td>Respiratory Rate (br.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>19.4</td>
<td>(3.0)</td>
<td>19.8</td>
<td>(2.7)</td>
<td>0.7</td>
<td>3.80</td>
<td>.93</td>
</tr>
<tr>
<td>Blood Lactate Conc. (mmol.L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.4</td>
<td>(0.4)</td>
<td>1.4</td>
<td>(0.4)</td>
<td>0.1</td>
<td>7.20</td>
<td>.94</td>
</tr>
<tr>
<td>Efficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross</td>
<td>16.78</td>
<td>(1.23)</td>
<td>16.92</td>
<td>(1.37)</td>
<td>0.32</td>
<td>1.91</td>
<td>.94</td>
</tr>
<tr>
<td>Net</td>
<td>21.98</td>
<td>(1.52)</td>
<td>22.50</td>
<td>(0.97)</td>
<td>0.56</td>
<td>2.51</td>
<td>.81</td>
</tr>
<tr>
<td>Tympanic Temperature (°C)</td>
<td>36.8</td>
<td>(0.6)</td>
<td>36.9</td>
<td>(0.06)</td>
<td>0.2</td>
<td>0.61</td>
<td>.84</td>
</tr>
<tr>
<td>Rating of Perceived Exertion</td>
<td>7.7</td>
<td>(0.9)</td>
<td>7.6</td>
<td>(1.08)</td>
<td>0.5</td>
<td>6.93</td>
<td>.71</td>
</tr>
</tbody>
</table>