UNIVERSITY OF
TECHNOLOGY SYDNEY

The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes

A thesis submitted for the degree

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By

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Certificate of Authorship and Originality of Thesis

I certify that the work contained in this thesis has not been previously submitted either in whole or in part for a degree at the University of Technology, Sydney or any other tertiary institution.

I also certify that the thesis has been written by me, Katie May Slattery. Any help that I have received in my research work and in the preparation of this thesis has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Katie Slattery

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Date Submitted

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'A man [women] is not an island', and PhD is not done alone. Many people have contributed to the completion of this thesis and I am sincerely thankful and appreciative to you all.

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Preface

This thesis for the degree of Doctor of Philosophy is in the format of published or submitted manuscripts and abides by the 'Procedures for Presentation and Submission of Theses for Higher Degrees – University of Technology, Sydney; Policies and Directions of the University'. All manuscripts included in this thesis are closely related in subject matter and form a cohesive research narrative.

Based on the research design and data collected by the candidate, three manuscripts have been submitted for publication and one manuscript has been accepted, in peer-reviewed journals. These papers are initially brought together by an *Introduction*, which provides background information, defines the research problem and the aim of each study. A *Literature Review* then follows to provide an overview of previous knowledge regarding the effect of intensified training periods and antioxidant supplementation on the oxidative, inflammatory and neuroendocrinological response to exercise. The body of the research is presented in manuscript form (*Chapter 3 to Chapter 6*), in a logical sequence following the development of research ideas in this thesis. Each manuscript outlines and discusses the individual methodology and the findings of each study separately. The *General Discussion* chapter provides an interpretation of the collective findings and practical applications from the series of investigations conducted. Finally, a *Summary and Recommendations* chapter is a synopsis of the research hypothesis tested and conclusions from each project. Based on these findings, directions for future research are suggested. Author-date reference style has been used throughout the document and the reference list is at the end of the thesis.

List of Articles Submitted for Publication

Refereed Journal Publications

- **Slattery, K.M.**, Wallace, L.K. and Coutts, A.J. (*under review*). The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: Implications of antioxidant supplementation on physiological adaption and performance during intensified physical training. *Sports Med*.

- **Slattery, K.M.**, Wallace L.K. and Coutts, A.J. (*in press*). Antioxidant intake of well-trained athletes during intensified physical training. *J Sports Med Phys Fit*.

- **Slattery, K.M.**, Wallace, L.K., Bentley, D.J. and Coutts, A.J. (*in press*). Effect of training load on simulated team sport match performance. *Appl Phys Nut Met*.

- **Slattery, K.M.**, Wallace, L.K., Bentley, D.J. and Coutts, A.J. (*under review*). Evaluating the effect of acute changes in training load on select endocrinological and oxidative damage markers in team sport players. *Eur J Appl Physiol.*

- **Slattery, K.M.**, Coutts, A.J., Wallace, L.K., Bentley, D.J., and Dascombe, B.J. (*under review*). The effect of N-acetylcysteine on cycling performance following intensified training in well-trained triathletes: a double blind randomised placebo controlled study. *Med Sci Sports Exerc.*

Conference Proceedings & Abstracts

- **Slattery, K.M.**, Wallace, L.K. and Coutts, A.J. (2006). *Nutritional practices of elite swimmers during an intensified training camp: with particular reference to antioxidants.* Poster presented at the Australian Conference of Science and Medicine in Sport, Sydney, Australia.

- Coutts, A.J., **Slattery, K.M.**, Wallace L.K., and Sirotic, A.C. (2007). *Influence of between-match training load on match running performance and markers of recovery in team sport athletes*. Paper presented at the Australian Conference of Science and Medicine in Sport, Adelaide, Australia.

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Statement of Candidate Contribution

The contribution of each author to the investigations undertaken as part of the thesis is outlined in Table A below.

Table A: Percentage contribution (%) of each author to the investigations conducted during the candidature

Author	Study 1			Study 2				Study 3				
	Katie Slattery	Lee Wallace	Aaron Coutts	Katie Slattery	Lee Wallace	David Bentley	Aaron Coutts	Katie Slattery	Lee Wallace	Ben Dascombe	David Bentley	Aaron Coutts
Research design	80%	10%	10%	75%			25%	80%			10%	10%
Ethics Application	80%	10%	10%	80%			20%	100%				
Grant Application					25%		75%	90%				10%
Therapeutic Goods Administration Approval Application								75%				25%
Subject recruitment		100%		50%	50%			100%				
Data collection	50%	50%		70%	10%	10%	10%	100%				
Data analysis	100%			90%			10%	100%				
Biochemical analysis				90%		10%		70%		30%		
Statistical analysis	50%		50%	80%			20%	85%				15%
Manuscript Preparation	100%			100%				100%				
Manuscript Revision	25%	25%	75%	30%		10%	60%	10%	10%	10%	20%	60%

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Abstract

Introduction: Exercise induces a stress reaction that initiates adaptive processes, which can be modified by intensive physical training and / or exogenous antioxidant supplementation. However, the optimal exercise training strategy and corresponding level of antioxidant support for positive adaptation remains unclear. Therefore, the overall aim of this thesis was to investigate the interactions between exercise-induced changes within the oxidative, inflammatory and neuroendocrinological systems and antioxidant supplementation on athletic performance during intensive physical training. Three separate studies were undertaken and reported in four manuscripts. **Study 1:** In Study 1, well-trained athletes (n = 23) completed a 4 day food record during a period of intensified physical training. Collectively, the participants consumed a sufficient dietary intake of antioxidants (vitamin A, C and E) according to the Australian recommendations. **Study 2**: Study 2 used a crossover experimental design to examine the effect of intensive physical training on oxidative damage, inflammation, hormonal disturbances and performance capacity. Participants (n = 7) completed a high-intensity intermittent running protocol following both a reduced (LOW) and intensive (HIGH) 4 day physical training period. The results demonstrated that HIGH physical training led to an increased amount of muscle damage, decreases in sprint velocity ($P < 0.001$) and a reduction in total distance covered ($P < 0.05$) during the high-intensity intermittent running protocol. HIGH physical training also induced a greater increase in oxidative damage (xanthine oxidase) markers 2 h post-exercise (paper 1). Neuroendocrinological measures (growth and thyroid hormones) were not altered by training-induced fatigue (paper 2). These findings suggest that 4 day HIGH training can impair high-intensity running performance and exacerbate oxidative damage. **Study 3:** Study 3 used a double blind randomised placebo-controlled crossover design to investigate the effect of 9 d oral N-acetylcysteine (NAC) supplementation (1200 mg/day) in eight well-trained triathletes. Changes in performance (cycle ergometer race simulation) and pre- to post-exercise biochemistry measures were taken to determine the ergogenic effect of NAC and associated reaction within the oxidative and inflammatory systems. It was demonstrated that oral NAC supplementation enhanced repeat sprint cycling performance via an improved redox balance and promoted adaptive processes in well-trained triathletes undergoing intensive physical training. NAC supplementation was also effective at blunting the inflammatory response to exercise. **Conclusion:** Collectively, this thesis provides novel information regarding the dose-response relationship between training-induced fatigue, antioxidant supplementation and athletic performance.

Keywords

Antioxidant

Fatigue

Inflammation

Intensified physical training

Muscle Damage

N-acetylcysteine

Nuclear factor – kappaB

Oxidative damage

Performance

Hormone

List of Abbreviations

8-OHdG	8-hydroxy-deoxyguanosine
AMP	adenosine monophosphate
AP-1	activating protein-1
AU	arbitrary units
CAT	catalase
Cd$_{max}$	maximum amount of conjugated dienes
CI	confidence interval
COX-2	cyclooxygenase
CR-10	category ratio 10
CV	coefficient of variation
d	Cohen's d effect size
DAG	diacylglycerol
DALDA	Daily Analysis of Life Demands for Athletes
F$_2$-isoprostane	15-isoprostane F$_{2t}$ concentration
FRAP	ferric reducing ability of plasma
FT$_3$	free triiodothyronine
FT$_4$	free thyroxine
g	Hedge's g effect size
GH	growth hormone
Gr	glutathione reductase
GPX	glutathione peroxidise
GSH	reduced glutathione
GSH:GSSG	reduced glutathione to glutathione ratio
GSSG	glutathione
HIGH	intensified training period
H$_2$O$_2$	hydrogen peroxide;
HSF	heat shock factor
HSP	heat shock protein
ICC	intra-class correlation
IκB	inhibitor -kappaB
IκK	inhibitor - kappaB kinase
IL-6	interlukin-6
iNOS	inducible nitric oxide synthase
ISAK	International Society for the Advancement of Kinanthropometry
JNK	c-Jun N-terminal kinases
LDH	lactate dehydrogenase
lipid-ox	lag time in lipid peroxidation
LOH	redox inert alcohol
LOOH	lipid hydroperoxide
LOW	low training load
Lp	length of lag phase
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MDA	malondialdehyde
MKK	MAP kinase kinase
MnSOD	manganese superoxide dismutase
mRNA	messengerRNA
η^2_p	partial eta squared
NAC	N-acetylcysteine

NADPH	nicotinamide-adenine dinucleotide phosphate
NF-κB	nuclear factor-kappaB
nm	nanometre
NMT	non-motorised treadmill
O$_2$	oxygen
ORAC	oxygen radical absorbance capacity
Oxhem	oxidatively modified heme
P	phosphorus
p50/65	subunits of NF-κB
PC	Protein carbonyls
PCr	phosphocreatine
PKC	protein C kinase
PKR	double-stranded RNA protein kinase
PLC	phospholipase
PPARγ	peroxisome-proliferators-activated receptor gamma
PPAR	peroxisome-proliferator-activated receptor
PUFA	poly unsaturated fatty acid
RDI	recommended daily intake
Redox	reduction-oxidation
R$_{max}$	maximum rate of oxidation
RPE	rating of perceived exertion
ROS	reactive oxygen species
Se	selenium
SOD	superoxide dismutase
SRM	Schoberer Rad Meßtechnik
T	training load period
TAC	total antioxidant capacity
TBARS	thiobarbituric acid-reactive substances
TE	typical error
TEAC	trolox-equivalent antioxidant capacity
TEM	technical error of measure
TEM%	percentage technical error of measure
TNF-α	tumor necrosis factor-α
TSH	thyroid stimulating hormone
TL	training load
UA	uric acid
XO	xanthine oxidase

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CHAPTER ONE

Introduction

1. Background

Exercise induces a systemic stress reaction, which initiates regulatory changes in biological systems to accommodate for the acute increase in work demands. This disruption in homeostatic balance also stimulates cellular signalling pathways for increases in gene expression and protein synthesis (Powers, Duarte et al. 2010). It is via this process that physical activity promotes numerous beneficial adaptations within the body to improve health and well-being (Radak, Chung et al. 2008). However, to maximise improvements in performance, athletes are required to complete successive bouts of strenuous exercise. These intensified periods of physical training can place excessive stress on biologic function, leading to fatigue and maladaptation (Meeusen, Duclos et al. 2006). It is currently unclear as to the exact training stimuli that will either positively or negatively affect physiological adaptation and performance capacity. An improved understanding of the mechanisms that underpin the adaptive processes can assist in the prescription of the most appropriate exercise stimuli to optimise athletic performance.

It has been proposed that the oxidative system is an underlying mediator of the training-induced adaptive response (Radak, Chung et al. 2008). The rapid increase in oxidant concentration at the onset of exercise initiates an increase in antioxidant enzyme expression and facilitates the mobilisation of exogenous antioxidants. However, this was not always the case. When the release of reactive oxygen species (ROS) during exercise was first identified, the increase was seen as a negative side-effect (Sjodin, Hellsten Westing et al. 1990). These elevations in ROS during strenuous exercise bouts could cause substantial oxidative damage to cellular structures, propagate the inflammatory response and lead to dysregulation within the neuroendocrine system (Tiidus 1998; Balon and Yerneni 2001). Nonetheless, closer investigation revealed that during moderate exercise bouts, the rise in ROS is easily controlled by the antioxidant system and a minimal amount of oxidative damage is incurred (Radak, Chung et al. 2008). Instead, through the process of reduction-oxidation (redox) signalling, exercise-induced increases in ROS act as molecular messengers to promote the transcriptional regulation of cellular growth and adaptation (Powers, Duarte et al. 2010). In fact, research has also highlighted that if the exercise-induced increases in ROS are blunted by antioxidant supplementation, the adaptive processes to training stimuli can be impaired (Ristow, Zarse et al. 2009). Antioxidant supplements have also been demonstrated to exert an acute ergogenic effect on performance (Reid, Stokic et al. 1994). It appears that a delicate balance exists between ROS release and antioxidant supplementation. Additional

research is required to further establish the dose-response relationship between physical training and antioxidant intake.

2. Research Problem

Many of the current concepts regarding the influence of redox regulation on physiological adaptation have arisen from animal based, *in vivo* or *in situ* experimental designs. When changes in the redox balance are investigated in well-trained athletes, the impact of intensive physical training and antioxidant supplementation on performance are less clear. This thesis aims to provide additional information concerning the antioxidant status of well-trained athletes and associated changes in the oxidative, inflammatory and neuroendocrinological systems.

3. Study Objectives

An applied research approach was used to examine the exercise-induced response of the oxidative, inflammatory and neuroendocrinological systems during intensified physical training. In addition, the effect of an individual's antioxidant status on training-induced stress was investigated to develop a greater understanding of the balance between physical training dose and antioxidant supplementation. It was anticipated that the results of these investigations would provide valuable insight into the way in which the body adapts to exercise and be relevant to athletes in a sporting setting. A series of three studies were conducted.

Study 1: Antioxidant intake of well-trained athletes during intensified physical training (Chapter 3)

Aim

To assess the nutritional antioxidant intake from whole foods in well-trained athletes.

Hypothesis

Athletes would meet the Australian recommended daily intake for antioxidant vitamins.

Significance

Previous research has identified that antioxidant supplementation may have a detrimental effect on performance, contribute to increases in oxidative damage / inflammation and suppress gene expression (Peternelj and Coombes 2011). However, athletes undergoing strenuous physical training periods (Palazzetti, Rousseau et al. 2004)

or consuming a low antioxidant diet (Watson, Callister et al. 2005) have also been shown to have an increased susceptibility to exercise-induced oxidative damage / inflammation. It was therefore important to establish an individual's dietary intake of antioxidant vitamins prior to advising upon exogenous antioxidant supplementation.

Study 2: Effect of acute changes in training load on select oxidative, inflammatory and neuroendocrinological markers and high intensity intermittent running performance in team sport players (Chapter 4 and Chapter 5)

Aim

To examine the impact of an acute increase in training load following a strenuous exercise bout on performance and exercise-induced changes within the oxidative, inflammatory and neuroendocrinological systems.

Hypothesis

An intensive training load following a strenuous bout of exercise would impair recovery and induce a state of acute fatigue. When a subsequent bout of strenuous exercise was completed, this state of fatigue would exacerbate performance decrements. Moreover, a significantly greater amount of oxidative damage, increases in inflammatory indicators and impairment of the neuroendocrinological response to exercise would occur.

Significance

It is commonplace for athletes to compete on a weekly basis. Insufficient recovery between these competitive performances may negatively affect physiological capacity (Ispirlidis, Fatouros et al. 2008). A greater understanding of the dynamics between performance and exercise-induced changes within the oxidative, inflammatory and neuroendocrinological systems may assist in monitoring fatigue levels. This information can then be used in the prescription of appropriate training stimuli to maintain fitness throughout the competitive season and facilitate optimal performance.

Study 3: The effect of N-acetylcysteine on cycling performance following intensified training in well-trained triathletes: a double blind randomised placebo controlled study (Chapter 6)

Aim

To determine the effect of a 9 d loading period with the antioxidant, N-acetylcysteine (NAC) on performance and exercise-induced changes within the oxidative and inflammatory systems following intensified physical training.

Hypothesis

Oral NAC supplementation would improve performance during a cycle ergometer race simulation. However, this performance improvement would be at the expense of adaptive responses [measured via activity of the transcription factor, nuclear factor – kappaB (NF-κB)] and may cause increased amounts of oxidative damage and inflammation.

Significance

Previous research has demonstrated that NAC supplementation can promote fatigue resistance in skeletal muscle by maintaining an optimal redox balance within contractile fibres (Reid, Stokic et al. 1994). Currently, there is limited evidence to suggest that oral NAC can improve performance in a practical sports setting (Cobley, McGlory et al. 2011). Further research is required to establish the efficacy and appropriate supplementation protocols for the use of NAC in well-trained athletes. Considering, antioxidant supplementation has also been demonstrated to blunt the redox mediation of exercise-induced adaptive processes (Ristow, Zarse et al. 2009), it is important to determine the impact of NAC supplementation on redox signalling pathways and associated disturbances in the oxidative / inflammatory systems. These findings will provide information on suitable recommendations for NAC supplementation in athletes to promote performance and physiological adaptation.

CHAPTER TWO

Literature Review

Slattery, K.M., Wallace, L.K. and Coutts, A.J. (*under review*). The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: Implications of antioxidant supplementation on physiological adaption and performance during intensified physical training. *Sports Med*.

Abstract

Exercise-induced increases in reactive oxygen species (ROS) act as intra-cellular messengers to regulate physiological adaptation. However, during periods of intensified physical training, ROS release may exceed the protective capacity of the antioxidant system and lead to dysregulation within the inflammatory and neuroendocrinological systems. Consequently, the ability of exogenous antioxidant supplementation to maintain the oxidative balance in states of exercise stress has been widely investigated. The aim of this review was to: (i) collate the findings of prior research on the effect of intensive physical training on oxidant-antioxidant balance; (ii) surmise the influence of antioxidant supplementation on the reduction-oxidation signalling pathways involved in physiological adaptation; (iii) conduct a brief systematic review to establish the potential of antioxidant supplements to exert an ergogenic effect on exercise performance; and, (iv) provide a synopsis on the interactions between the oxidative, inflammatory and neuroendocrinological response to exercise stimuli. Based on prior research it is evident that ROS are an underlying aetiology in the adaptive process. An excessive release of ROS has commonly been reported in athletes in an exercise-induced fatigued state. The systematic analysis revealed that the antioxidants, N-acetylcysteine and quercetin, can exert a small ergogenic effect on athletic performance. Nonetheless, the impact of antioxidant supplementation on physiological adaptation remains unclear. Equivocal results have been reported on the impact of antioxidant supplementation on exercise-induced gene expression. Further research is required to establish whether the interference of antioxidant supplementation consistently observed in animal-based and *in vivo* research extends to a practical sports setting. Moreover, the varied results reported within the literature may be due to the hormetic response of oxidative, inflammatory and neuroendocrinological systems to an exercise stimulus. The collective findings suggest that intensified physical training places substantial stress on the body, which can manifest as an adaptive or maladaptive physiological response. Additional research is required to determine the efficacy of antioxidant supplementation to minimise exercise-stress during intensive training and promote an adaptive state.

The Stress-Response to Exercise

Physical training improves athletic performance in a stimulus-response manner, dependant on the volume, intensity and frequency of each exercise bout (Busso 2003). Alike other stressors, exercise perturbs the homeostatic balance within the body (Radak, Chung et al. 2005). In order to retain homeostasis, an up-regulation of biological systems occurs, which exceeds the previous level of function. It is through this process that adaptation to stress takes place (Selye 1956). Imbalances between exercise-induced stress and recovery may cause these physiological adaptations to be impaired, leaving an individual in a maladaptive state where performance may stagnate or even decline (Meeusen, Duclos et al. 2006). Indeed, prolonged periods of intensified physical training may result in an accumulation of fatigue leading to short-term (non-functional overreaching) and long-term (overtraining) decrements in performance capacity (Meeusen, Duclos et al. 2006). Excessive exercise may also contribute to disturbances in biological function within the metabolic (Snyder 1998; Petibois, Cazorla et al. 2003), neuroendocrinological (Steinacker, Lormes et al. 2004), oxidative (Tiidus 1998), physiological (Halson, Bridge et al. 2002), psychological (Kellmann 2010) and immunological (Smith 2004) systems. Collectively, a dysregulation in these systems may contribute to the signs and symptoms presented in overreached / overtrained athletes and ultimately lead to unwanted reductions in performance. Despite extensive research, the underlying patho-physiological mechanism and physical training load, which may either maximise physiological adaptation or cause a state of maladaptation, are yet to be fully understood.

An increased understanding of the multi-factorial relationship between the oxidative, inflammatory and endocrinological systems during intensified training periods will assist coaches, sports scientists and athletes to better elicit the desired physiological adaptations to achieve peak performance. The purpose of this review was to examine the interactions between the oxidant-antioxidant balance, inflammation and neuroendocrine regulation during periods of intensified training to modulate physiological adaption. Relevant literature was collated using a combination of keyword search strings (exercise, physical training, overreaching, overtraining, fatigue, performance, adaptation, oxidative stress, reactive oxygen species, free radical, antioxidant, redox, gene expression, homeostasis, hormesis, immune, inflammation, hormonal, endocrine) in the Google Scholar, Sports Discus and United States National Library of Medicine Pubmed databases. Key papers were identified, cross-referenced and refined to focus on investigations in the athletic population.

Free Radical Biochemistry and Exercise

Redox Reactions and Adaptation to Exercise

Free radicals are unstable atoms and molecules with one or more unpaired electron (Halliwell and Gutteridge 1999). Free radicals readily participate in reduction-oxidation (redox) reactions resulting in a reduction of the free radical and the subsequent oxidisation of the molecule (Droge 2002). The majority of free radicals are either oxygen or nitrogen based and are referred to as reactive oxygen species (ROS) and reactive nitrogen species (Droge 2002). An increase in ROS concentration acts as a biological messenger, initiating cellular signalling cascades via the process of electron transfer to promote adaptive responses within the body (Powers, Duarte et al. 2010). However, the oxidisation of molecules, if uncontrolled can also lead to lipid, protein and DNA damage, resulting in impaired cellular function (Alessio, Hagerman et al. 2000). Consequently, the body has an elaborate antioxidant defence system to minimise this oxidative damage from occurring by converting ROS to less reactive molecules or by removing molecules that may promote further oxidative reactions (Thomas 2000). The redox balance is easily disturbed by the rapid exercise-induced release of ROS from the blood and skeletal muscle (McArdle, Pattwell et al. 2001; Nikolaidis and Jamurtas 2009). Therefore, the oxidative response to exercise has been widely researched in relation to both health and athletic performance.

The process of gene expression and activation of DNA transcription initiates the synthesis of new proteins in response to a stressor (Allen and Tresini 2000). Exercise-induced alterations in redox status play an important role in the modulation of numerous genetic transcription pathways, which promote adaptations to physical training (Figure 2.1) (Allen and Tresini 2000; Powers, Duarte et al. 2010). Many of these adaptations are designed to protect the cellular structure from further oxidative insults (Radak, Chung et al. 2008). ROS release at the onset of exercise initiates redox signalling cascades to improve cellular antioxidant protection via the increased gene transcription of multiple antioxidant enzymes (Ji 2008). Individuals that regularly participate in physical activity display an augmented endogenous antioxidant enzyme capacity (Berzosa, Cebrian et al. 2011) and an increased tolerance to exercise-induced oxidative stress (Brites, Evelson et al. 1999; Cazzola, Russo-Volpe et al. 2003; Dekany, Nemeskeri et al. 2005; Pittaluga, Parisi et al. 2006). Redox signalling also plays a central role in the up-regulation of the mitogen-activated protein kinase (MAPK) family. The MAPK pathway modulates biological processes including the growth and differentiation of skeletal muscle fibres, glucose transport and angiogenesis in a

redox dependent manner (Kramer and Goodyear 2007). Additionally, ROS signalling can promote activation of the nuclear factor-kappa B (NF-κB) pathway that directly impacts the expression of genes involved in the acute-phase response, apoptosis, muscle fibre regeneration and metabolism (Pahl 1999). Similarly, ROS can mediate the heat shock protein (HSP) response to exercise (Schlesinger 1990). Increases in HSP expression are necessary to maintain cellular function in subsequent bouts of stressful exercise by acting as intra-cellular chaperones for protein remodelling and synthesis (Schlesinger 1990). Furthermore, mitochondrial biogenesis is regulated by the redox sensitive transcription of peroxisome proliferators activated receptor γ coactivator-1α (PGC-1α) (Hood 2009; Irrcher, Ljubicic et al. 2009). These findings emphasise the importance of the redox homeostasis in promoting physiological adaptation and improved performance capacity following an exercise stimulus.
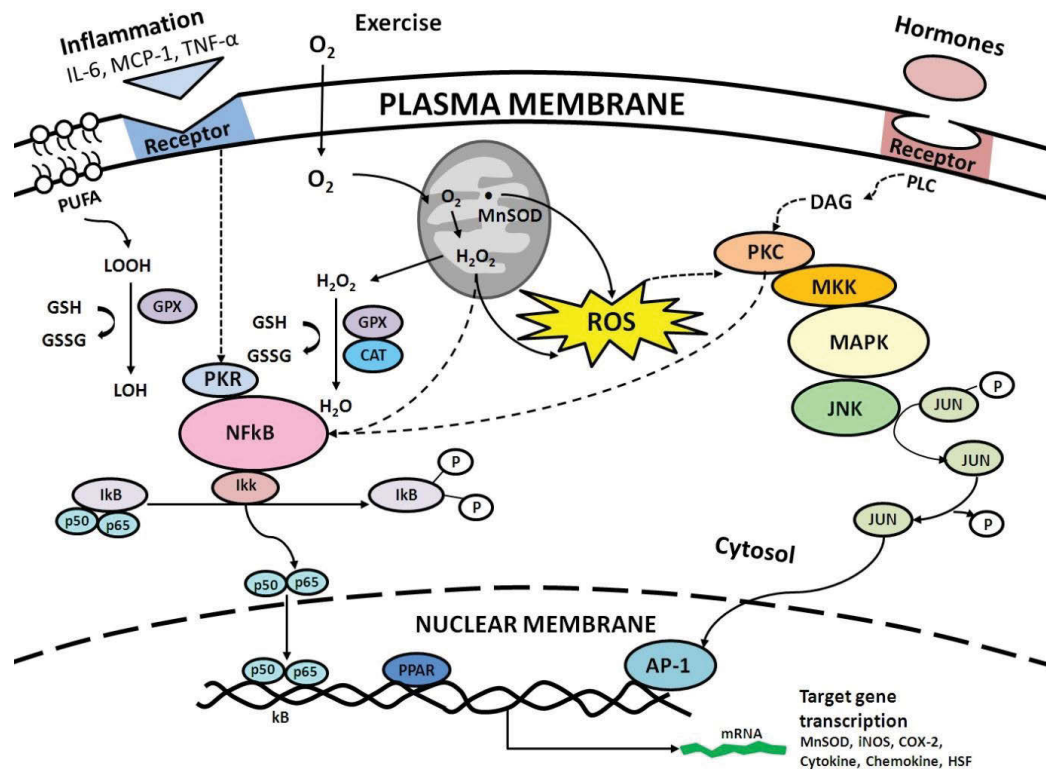
Figure 2.1: A schematic diagram illustrating the redox signalling pathways for skeletal muscle adaptation [adapted from Ji (2008)].

AP-1 = activating protein-1; **CAT** = catalase; **COX-2** = cyclooxygenase; **DAG** = diacylglycerol; **GPX** = glutathione peroxidise; **GSH** = reduced glutathione; **GSSG** = glutathione; **H$_2$O** = water; **H$_2$O$_2$** = hydrogen peroxide; **HSF** = heat shock factor; **IκB** = inhibitor -κB; **IκK** = IκB kinase; **IL-6** = interlukin-6; **iNOS** = inducible nitric oxide synthase; **JNK** = c-Jun N-terminal kinases; **LOH** = redox inert alcohol; **LOOH** = lipid hydroperoxide; **MAPK =** mitogen activated protein kinase; **MCP-1 =** monocyte chemoattractant protein-1; **MKK** = MAP kinase kinase; **MnSOD =** manganese superoxide dismutase; **mRNA** = messenger RNA; **NF-κB** = nuclear factor-kappaB; **O$_2$** = oxygen; **P** = phosphorus; **p50/65** = subunits of NF-κB; **PKC** = protein C kinase; **PKR** = double-stranded RNA protein kinase; **PLC** = phospholipase; **PPAR** = peroxisome-proliferator-activated receptor; **PUFA** = poly unsaturated fatty acid; **ROS** = reactive oxygen species and **TNF-α** = tumor necrosis factor-α.

The process of redox signalling is complex and an over or under production of ROS can result in a state of maladaptation (Radak, Chung et al. 2008). Low concentrations of ROS are necessary for proper regulation of cellular function and adaptation to exercise stress (Lachance, Nakat et al. 2001).   However, strenuous exercise can result in an excessive production of ROS and result in oxidative damage to cellular lipids, proteins and DNA, a condition commonly referred to as oxidative stress (Halliwell and Gutteridge 1999). During periods of oxidative stress, the redox homeostatic point may readjust and impair the redox signalling pathways (Droge 2002). It is likely that during intensified physical training the redox homeostasis will be disrupted, inducing a state of chronic oxidative stress which may contribute to a reduction in athletic performance capacity (Itoh, Ohkuwa et al. 2000; Droge 2002; Palazzetti, Richard et al. 2003; Finaud, Scislowski et al. 2006).

Intensified Physical Training Loads and Oxidative Stress

In well-periodised physical training programs, an individual's antioxidant defence system is capable of controlling exercise-induced oxidant production to promote an optimal redox balance for muscle contractile function and physiological adaptation (Pittaluga, Parisi et al. 2006; Vollaard, Cooper et al. 2006). Numerous investigations have reported reduced oxidative disturbance following short-term training interventions, indicating that the individual is adapting appropriately to the imposed physical training load (Brites, Evelson et al. 1999; Svensson, Ekblom et al. 2002; Nikolaidis, Paschalis et al. 2007; Campbell, Gross et al. 2010). In contrast, unaccustomed, high intensity, prolonged or strenuous exercise can place a considerable strain on the antioxidant system and lead to a state of oxidative stress (Alessio, Hagerman et al. 2000; Mastaloudis, Leonard et al. 2001). A sustained increases in oxidant production may lead to disturbances in redox homeostasis potentially leading to physiological maladaptation (Tanskanen, Atalay et al. 2010). In fact, oxidative stress has been suggested to be one possible factor underlying non-functional overreaching and overtraining in athletes.

Studies examining the oxidant-antioxidant balance in both endurance and team sport athletes have identified a possible relationship between training load and the level of oxidative stress (Santos-Silva, Rebelo et al. 2001; Palazzetti, Richard et al. 2003; Finaud, Scislowski et al. 2006; Margonis, Fatouros et al. 2007; Tanskanen, Uusitalo et al. 2011). These investigations have shown that during intensified training periods, athletes may display increased levels of oxidative damage and a blunted antioxidant response to exercise. For instance, Finaud et al. (2006) reported significantly higher oxidative damage (67% increased in conjugated diene oxidation) and impaired antioxidant protection (8.7% decrease in plasma vitamin E), in 17 male professional rugby players during a 4 wk intensive training period. Similarly, post-exercise measures of oxidative damage (lipid peroxidation) were elevated and antioxidant status was reduced in nine well-trained male triathletes following a 4 wk overtraining period (Palazzetti, Richard et al. 2003). Collectively, these findings suggest that during periods of excessive physical training, the antioxidant system may become overwhelmed by repeated bouts of exercise-induced oxidant production. This decrease in antioxidant capacity may leave athletes more susceptible to oxidative damage and lead to a chronic state of oxidative stress.

Not all investigations have demonstrated this concise inter-relationship between training load, oxidative stress and antioxidant defences. Tanskanen et al. (2011) reported a significant increase in oxidative stress (reduced glutathione:glutathione ratio) and lipid peroxidation at rest in 11 overreached military personnel without observing any changes in resting or post-exercise measures of protein oxidation or antioxidant capacity. Other well-designed investigations have also shown no dose-response effect of intensified training on the redox balance (Palazzetti, Rousseau et al. 2004; Vollaard, Cooper et al. 2006; Teixeira, Valente et al. 2009). The inconsistency of previous research may be attributed to the relatively low number of studies focussed on athletic populations, the different biochemical markers used to assess redox balance and variations in performance measures, training and testing procedures. A summary of these previous investigations is provided in Table 2.1. These methodological disparities make it difficult to synthesise the impact of intensified training on oxidative stress. However, there is evidence to suggest that ROS are an underlying aetiology in exercise-induced disturbances in homeostasis. The majority of these studies show that whilst a moderate level of ROS are a necessary prerequisite for adaption to exercise (Bailey, Davies et al. 2001), sustained increases in oxidant production are likely to impair competitive performance (Schippinger, Wonisch et al. 2002; Palazzetti, Richard et al. 2003; Howatson, McHugh et al. 2010; Tanskanen, Atalay et al. 2010). At present, the threshold where oxidative stress becomes detrimental, as opposed to beneficial to performance remains unclear. Therefore, further research examining the relative impact of increased ROS production on redox signalling pathways and the regulation of other physiological responses (i.e. neuroendocrinological) to exercise is warranted.

Table 2.1: Summary of previous investigations on oxidative stress measures following intensified training.

Author	Subjects	Intensified training period	Exercise bout	Resting measures following the intensified training		Post-exercise measures following the intensified training		Findings
				Antioxidant	Oxidant	Antioxidant	Oxidant	
Acute increase in training load								
Okamura (1997)	well-trained male runners (n=10)	8 d training camp (30 km/day)		↑ plasma vitamin E, ↔ plasma β-carotene	↑ urinary 8-OHdG, ↔ lymphocyte 8-OHdG, ↑ plasma MDA			Repeated exercise augments oxidative stress and results in oxidative DNA damage.
Shing (2007)	highly trained male cyclists (n=8)	3 d consecutive high-intensity cycling		↑ plasma TAC, ↔ erythrocyte SOD or GPX	initial ↑ plasma MDA then ↓	initial ↑ plasma TAC then ↓, ↔ erythrocyte SOD or GPX	initial ↑ plasma MDA then ↓	Post-exercise measures of oxidative damage were attenuated with consecutive days of high-intensity exercise.
Radak (2000)	well-trained male supra-marathon runners (n=5)	4 d racing period (d1-93 km, d 2-120 km, d 3-56 km, d 4-59 km)			↑ urinary 8-OHdG on d 1,2,3 that returned to baseline on d 4 of the race			Extreme exercise-induced stress does not propagate an increase in oxidative DNA damage.
Rowlands (2011)	well-trained runners (female n=4; male n=16)	4 d team running relay (mean dist per person 119.5 km)				↔ plasma TAC	↑ urinary F_2-isoprostane, ↔ urinary 8-OHdG	Elevated markers of oxidative damage were observed following the multi-stage run race.
Overreaching								
Palazzetti (2003)	well-trained male triathletes (n=9)	4 wk normal training, 4 wk overload training	Duathlon race simulation	↔ plasma TAC, ↔ plasma SOD, ↑ plasma GPX	↔ whole blood GSSG, ↔ whole blood GSH:GSSG, ↔ plasma MDA	↓ plasma TAC, ↔ plasma SOD, ↑ plasma GPX	↑ whole blood GSSG, ↑ whole blood GSH:GSSG, ↑ plasma MDA	No change in oxidant-antioxidant measures at rest. Reduced antioxidant response and increased oxidative stress post-exercise following intensified training periods.
Palazzetti (2004)	well-trained triathletes (n=10)	42% increase in TL for 4 wk	Duathlon race simulation	↔ serum Se; ↔ plasma ascorbic acid or α-tocopherol; ↔ erythrocyte SOD, ↑ erythrocyte and plasma GPX	↔ whole blood GSH:GSSG, ↔ plasma MDA	↔ serum Se, ascorbic acid or α-tocopherol; ↔ erythrocyte SOD; ↑ erythrocyte and plasma GPX	↔ whole blood GSH:GSSG, ↔ plasma MDA	No difference in the oxidative response to a duathlon race simulation during intensified training compared to normal training loads.
Vollaard (2006)	well-trained male triathletes (n=8)	8 wk cross over training program with periods of intensified and recovery	Cycle ergometer test		↔ whole blood GSSG, ↔ whole blood GSH:GSSG, ↔ whole blood OxHm		↔ whole blood GSSG, ↔ whole blood GSH:GSSG, ↔ whole blood OxHm	No change was observed in oxidative stress between intensified training and recovery.
Margonis (2007)	recreationally trained men (n=12)	4 x 3 wk training periods (T 1 low, T 2 medium, T 3 intensified, T 4 low)	T 3 compared to T 1	↓ plasma TAC, ↑ plasma GPX, ↑ plasma CAT	↑ urinary F_2-isoprostane, ↑ whole blood GSSH:GSH, ↑ serum PC, ↑ plasma MDA			Changes in biomarkers of oxidative stress reflected fluctuations in training load and performance.

Table 2.1: (continued)

Author	Subjects	Intensified training period	Exercise bout	Pre-exercise measures following the intensified training		Post-exercise measures following the intensified training		Findings
				Antioxidant	Oxidative	Antioxidant	Oxidative	
Overreaching								
Tanskanen (2011)	military trained males (n=11)	8 wk military basic training		↔ plasma ORAC	↑ plasma GSSG, ↑ plasma GSH:GSSG, ↑ plasma MDA, ↔ plasma PC	↔ plasma ORAC	↔ plasma GSSG, ↔ plasma GSH:GSSG, ↔ plasma MDA, ↔ plasma PC	Intensified physical training increased oxidative stress at rest and following a 45 min sub-maximal run in subjects that were determined to be overreached.
Time course changes over a season								
Santos-Silva (2001)	competitive swimmers (n=40), active adolescents (n=42)	Swimmers; TL-20 hr/wk compared to physically active;TL-2-4 h/wk		↔ plasma TAC	↑ plasma TBARS			Athletes completing high training loads displayed a greater amount of oxidative damage compared to matched sedentary controls.
Schippinger (2002)	well-trained American football players (n=8)	Resting blood measures throughout the competitive season	Highest TL compared to lowest	↔ plasma β-carotene, ↔ plasma α-tocopherol, ↑ plasma ascorbate	↑ plasma total peroxides, ↔ plasma lipid-ox			An increased level of oxidative stress was observed throughout the competitive season.
Finaud (2006)	professional rugby union players (n=17)	Resting blood measures throughout the competitive season	Highest TL compared to lowest	↔ plasma TAC, ↓ plasma Lp, ↓ plasma α-tocopherol, ↑ plasma UA	↑ R_{max} ↔ CD_{max}			Increased levels of oxidative stress and reduced efficiency of the antioxidant defence system were observed during intense periods of physical training and competition.
Teixeira (2009)	elite kayakers (female; n=3, male; n=6)	T 1-560 min/wk, T 2-650 min/wk, T 3- 410 min/wk	Highest TL compared to lowest	↔ plasma Gr, ↓ plasma GPx, ↑ plasma SOD, ↑ plasma α-tocopherol, ↔ plasma β- carotene,	↓ plasma TBARS			No evidence of an increased disturbance in redox homeostasis was observed when greater training load was completed.
Overtraining								
Tanskanen (2010)	overtrained athletes (female; n=2, male; n=5)	Comparison pre / post exercise to control athletes	Cycle ergometer step test to exhaustion	↑ plasma ORAC	↑ plasma PC, MDA	↔ plasma ORAC	↑ plasma PC, ↔ plasma MDA	Blunted antioxidant response to exercise and an increased basal level of oxidative stress.

8-OHdG = 8-hydroxy-deoxyguanosine; **d** = day; **CAT** = catalase; **Cd**$_{max}$ = maximum amount of conjugated dienes; **Gr** = glutathione reductase; **GPX** = glutathione peroxidise; **GSH** = reduced glutathione; **GSH:GSSG:** = reduced glutathione:gluthathione ratio; **GSSG** = glutathione; **lipid-ox** = lag time in lipid peroxidation; **Lp** = length of lag phase; **MDA** = malondialdehyde; **min** = minute; **ORAC** = oxygen radical absorbance capacity; **Oxhem** = oxidatively modified heme; **PC** = Protein carbonyls; **R**$_{max}$ = maximum rate of oxidation; **Se** = selenium; **SOD** = superoxide dismutase; **T** = training load period; **TAC** = Total antioxidant capacity; **TBARS** = thiobarbituric acid-reactive substances; **TEAC** = Trolox-equivalent antioxidant capacity; **TL** = training load; **wk** = week and **UA** = uric acid

Antioxidant Supplementation and Adaptation to Exercise

An antioxidant is a substance which can delay or prevent the oxidation of other substrates (Halliwell, Aeschbach et al. 1995). Antioxidants are present in both the intra- and extra-cellular matrix and form a complex defence system to protect cells and tissues against excessive oxidative damage to lipids, proteins and nucleic acids (Halliwell, Aeschbach et al. 1995). During exercise, the elevated oxidant production in skeletal muscle is controlled by a synergistic response between the endogenous antioxidant system and the antioxidant vitamins and minerals consumed as part of a well-balanced diet (Watson, MacDonald-Wicks et al. 2005). The antioxidant system readily adapts to physical training stimuli to counterbalance the potentially harmful effects of excessive exercise–induced ROS and to maintain the redox controlled signalling pathways (Ji 2008). However, it has been suggested that as athletes produce an increased amount of oxidative stress during repeated bouts of exercise, they may require a greater amount of exogenous antioxidants to maintain health and improve performance (Palazzetti, Rousseau et al. 2004; Watson, Callister et al. 2005).

Well-trained athletes generally have an enhanced antioxidant capacity and are less susceptible to exercise induced redox perturbations compared to the sedentary population (Brites, Evelson et al. 1999; Cazzola, Russo-Volpe et al. 2003; Dekany, Nemeskeri et al. 2005; Pittaluga, Parisi et al. 2006; Melikoglu, Kaldirimci et al. 2008; Falone, Mirabilio et al. 2010). However, factors such as a sudden increase in physical training load (Palazzetti, Richard et al. 2003; Palazzetti, Rousseau et al. 2004; Tanskanen, Uusitalo et al. 2011), exposure to altitude / hypoxic conditions (Bailey, Davies et al. 2001; Pialoux, Brugniaux et al. 2010), prolonged strenuous exercise (Mastaloudis, Leonard et al. 2001; Aguilo, Tauler et al. 2005; Rowlands, Pearce et al. 2011), pollution (Gomes, Allgrove et al. 2011) or an insufficient dietary intake of antioxidants (Farajian, Kavouras et al. 2004; Paschoal and Amancio 2004; Rousseau, Hininger et al. 2004) may exceed an individual's antioxidant defences to buffer oxidant production. Insufficient antioxidant protection may then contribute to elevated oxidative stress levels, altered neuroendocrine regulation, excessive skeletal muscle damage and/or an inability to adapt to continued physical training stimuli [for comprehensive reviews see (Tiidus 1998; Duntas 2005; Vollaard, Shearman et al. 2005; Peake, Suzuki et al. 2007; Fragala, Kraemer et al. 2011)]. Previous research has also identified that well-trained athletes may be at an increased risk of oxidative damage due to an insufficient dietary intake of antioxidants (Rousseau, Hininger et al. 2004). Consequently, the use of exogenous antioxidant supplementation to maintain antioxidant status has been extensively researched. There is

evidence which supports the ability of antioxidant compounds to blunt the oxidative (Sacheck, Milbury et al. 2003; Morillas-Ruiz, Zafrilla et al. 2005; Di Giacomo, Acquaviva et al. 2009; Lamprecht, Oettl et al. 2009; Arent, Pellegrino et al. 2010; Chang, Hu et al. 2010; Howatson, McHugh et al. 2010; Bowtell, Sumners et al. 2011; Diaz-Castro, Guisado et al. 2011), inflammatory (Phillips, Childs et al. 2003; Vassilakopoulos, Karatza et al. 2003; Chang, Hu et al. 2010; Howatson, McHugh et al. 2010; Diaz-Castro, Guisado et al. 2011; Nishizawa, Hara et al. 2011; Skarpanska-Stejnborn, Pilaczynska-Szczesniak et al. 2011) and hormonal (Peters, Anderson et al. 2001; Peters, Anderson et al. 2001; Davison, Gleeson et al. 2007; Teixeira, Valente et al. 2009) response to exercise. Indeed, 5 d of supplementation with tart cherry juice reduced oxidative damage [thiobarbituric acid-reactive substances (TBARS) and protein carbonyls] and markers of muscle inflammation [interlukin-6 (IL-6) and C-reactive protein (CRP)] 48 h post-marathon in 20 well-trained runners (Howatson, McHugh et al. 2010). Similar reductions in post-exercise measures of oxidative stress (F_2-isoprostanes) and muscle damage [creatine kinase (CK)] have been observed in 12 collegiate soccer players who consumed a mixture of antioxidant substances for a 3 wk period compared to a placebo (Arent, Pellegrino et al. 2010). These findings support the hypothesis that antioxidant supplementation may assist athletes to better cope with intensified training periods and prevent reductions in performance capacity during strenuous exercise (Reid, Stokic et al. 1994; Palazzetti, Rousseau et al. 2004; Watson, Callister et al. 2005).

Due to the reported protective effects of antioxidants, many athletes habitually take antioxidant supplements (Dascombe, Karunaratna et al. 2010). However, exogenous antioxidant compounds may prevent or reduce useful adaptive processes to exercise from occurring, especially if taken in high doses (Jackson, McArdle et al. 1998; Gomez-Cabrera, Domenech et al. 2008). Indeed, administration of 1000 mg/day vitamin C and 400 IU/day of vitamin E in 19 untrained and 20 active males has been shown to inhibit training-induced increases in skeletal muscle protein concentrations of transcription factors involved in mitochondrial biogenesis (PGC-1α), insulin sensitivity [peroxisome proliferator activated receptor gamma (PPARγ)] and a reduced mRNA concentration of antioxidant enzymes (glutathione peroxidise and superoxide dismutase) when compared to a placebo (Ristow, Zarse et al. 2009). Exercise-induced increases in skeletal muscle and lymphocyte HSP expression can also be impeded following antioxidant supplementation (Khassaf, McArdle et al. 2003; Fischer, Hiscock et al. 2006). Moreover, depending on the dosage and site of activity, certain antioxidants exert a pro-oxidant effect. As demonstrated by the association

of large doses of vitamin C (12.5 mg/kg body weight) with higher levels of exercise-induced oxidative damage and impaired recovery of muscle function (Childs, Jacobs et al. 2001). Based on these findings, it is currently recommended that rather than using supplements, physically active individuals should focus on consuming a well-balanced diet that includes a variety of high-antioxidant foods (Thompson, Heimendinger et al. 1999; Margaritis and Rousseau 2008).

An attenuation of redox signalling and adaptation with antioxidant supplementation has not been consistently shown during whole body exercise in human subjects (Chang, Hu et al. 2010; Nieman, Williams et al. 2010; Funes, Carrera-Quintanar et al. 2011; Petersen, McKenna et al. 2011). Whilst N-acetylcysteine (NAC) infusion blocked the post-exercise phosphorlyation of c-Jun N-terminal kinase, no effect was observed within the NF-κB or MAPK pathways, following a cycle ergometer test in eight endurance trained men (Petersen, McKenna et al. 2011). Similarly, quercetin supplementation (1000 mg/day) promoted skeletal muscle mRNA expression of genes involved in mitochondrial biogenesis in 26 previously untrained males during a 2 wk physical training period (Nieman, Williams et al. 2010). The additional quercetin also induced a greater increase in muscle mitochondrial DNA (4.1% increase) compared to the placebo trial (6.0% decrease) (Nieman, Williams et al. 2010). Indeed, whilst suppression of transcription factor activation with antioxidant supplementation have been consistently demonstrated in animal and *in vivo* investigations (Gomez-Cabrera, Borras et al. 2005; Silveira, Pilegaard et al. 2006; Gomez-Cabrera, Domenech et al. 2008), it is more difficult to establish similar results in human subjects during periods of physical training.

Further research is necessary to determine the ability of antioxidant supplementation to blunt exercise-induced adaptation in athletes as previous findings are equivocal and inconclusive (Williams, Strobel et al. 2006). This may be due to the numerous confounding variables including varying experimental designs, supplementation protocols (antioxidant compound, dosage, length of supplementation period), modes of exercise and previous training history. There is also a paucity of research conducted in the athletic population, focusing on the resultant effect of redox mediated gene expression on performance capacity. Nonetheless, these findings highlight the precarious balance within the redox signalling processes. It is likely that both an insufficient and an excessive amount of exogenous antioxidant consumption will have a negative effect on the adaptive process. Therefore, the

consumption of supra-nutritional antioxidant supplements may need to be periodised to provide additional support during periods of increased exercise-stress or to gain a potential ergogenic effect during competitive performances.

Effect of Antioxidant Supplementation on Performance

Overview

Previous experiments have shown that the direct administration of antioxidants such as NAC, dithiothreitol and L-2-oxothiazolidine-4-carboxylate can reduce the losses in contractile function associated with high levels of ROS production in isolated myofibres (Moopanar and Allen 2006; Ferreira, Gilliam et al. 2009). It is therefore plausible that exogenous antioxidant supplementation may delay fatigue within skeletal muscle and enhance performance in individuals during whole body exercise. To date, relatively few investigations have focused on the ergogenic potential of antioxidants in a sports setting. Moreover, these studies have produced conflicting results regarding the effect of exogenous antioxidant consumption on performance capacity (Peternelj and Coombes 2011). The purpose of the following section was to systematically review previous research conducted on the area of antioxidant supplementation and performance.

Search Strategy and Inclusion Criteria

An initial literature search was conducted and cross referenced using Endnote X4 Software (X4.0.1, Thomson Reuters, CA) within the Google Scholar, Sports Discus and US National Library of Medicine Pubmed databases using a variety of relevant keyword search strings (exercise, performance, athletic performance, ergogenic, antioxidant, vitamin C, ascorbic acid, vitamin E, α-tocopherol, β-carotene, N-acetylcysteine, polyphenols, quercetin, coenzyme Q_{10} and flavanoids). There were 513 articles were identified. The search was then delimited to crossover, randomised, placebo-controlled research designs in human subjects. Moreover, research which reported changes in the physiological response to exercise, as opposed to a measure of performance, was also excluded. This criteria yielded 31 published papers which were then further categorised according to the participant's training status, antioxidant compound [polyphenols (quercetin, epigallocatechin-3-gallate, ecklonia cava and grape extract), NAC, coenzyme Q_{10} and supplements containing a combination of nutricient antioxidant vitamins and minerals (AOX mixture)], loading period (acute; single dose −1 d,

short-term; 2–10 d and prolonged; > 10 d) and exercise type (aerobic or resistance). Training status was recorded in accordance with the participant description used in the article. The articles that were included in the review process had a total subject number of 431 (range 4 to 30) and were conducted on subjects with a mean age of 27 y (range 20 – 74 y) and mean $\dot{V}O_2$peak of 53.1 mL·kg^{-1}·min^{-1} (range 39.0 – 72.3 mL·kg^{-1}·min^{-1}). The mean quality of reporting for the articles reviewed was calculated as 60 ± 8%, according to the CONSORT guidelines (Schulz, Altman et al. 2010).

To provide a medium for comparison between the various exercise modalities, Hedges' g (g) was used to calculate a standardised mean difference between performance following the antioxidant and the placebo conditions (Hedges and Olkin 1985). Specifically, the difference between the mean of the performance measures following each treatment (antioxidant and placebo) was divided by the pooled standard deviation and then adjusted for sample size bias. The magnitude of Hedges' g effects were interpreted using thresholds of < 0.199 (trivial), 0.2 - 0.6 (small), 0.601 - 1.2 (moderate), 1.201 - 2.0 (large), and greater than 2.01 (very large). Statistical software (STATISTICA 8.0, Tulsa, USA) was used to calculate mean effect sizes and 90% confidence intervals (CI).

The Ergogenic Effect of Antioxidant Supplements

The systematic analysis demonstrated that the overall ergogenic effect of antioxidant supplementation on performance capacity in the 31 studies was small (g = 0.59; CI 0.36, 0.82). Further analysis on each separate antioxidant compound category revealed a moderate effect on performance with NAC (n = 9; g = 0.88; CI 0.47, 1.29) and polyphenol (n = 9; g = 0.62; CI 0.19, 1.05) supplementation. Small effects of supplementation were observed on performance for both coenzyme Q_{10} (n = 4; g = 0.57; CI -0.07, 1.22) and when AOX mixtures (n = 9; g = 0.23; CI -0.20, 0.82) were examined (Figure 2.2). When the effect sizes were calculated according to supplement loading period, results demonstrated that an acute protocol (g = 0.92; CI 0.56, 1.28) was the most effective in producing an ergogenic effect compared to short-term (g = 0.42; CI -0.06, 0.89) and prolonged (g = 0.37; CI 0.02, 0.72) supplementation periods. Antioxidant supplementation was shown to induce greater improvements in resistance-based performance tasks (g = 1.09; CI -0.58, 1.60) compared to aerobic performance capacity (g = 0.47; CI 0.23, 0.72). Finally, a small effect of antioxidant supplementation was observed in physically active (g = 0.43; CI 0.06, 0.80) and well-trained

(g = 0.50; CI 0.15, 0.86) subjects compared to a moderate effect in healthy untrained individuals (g = 1.02; CI 0.53, 1.50).
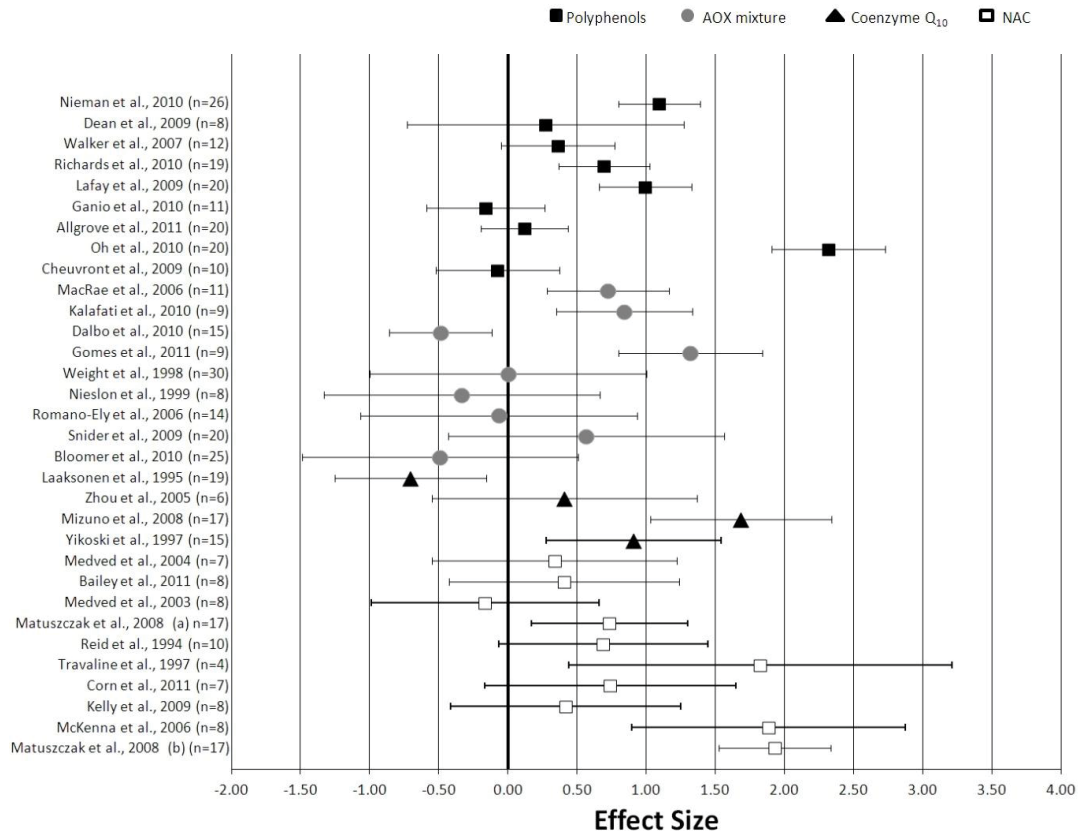
Figure 2.2: The effect size (Hedges g ± CI 90%) of antioxidants to improve exercise performance.

The results of the systematic review showed that antioxidant supplements could have a small positive effect on athletic performance. This is in agreement with a recent meta-analysis which reported an ~3% improvement in endurance performance following supplementation with the polyphenol compound, quercetin (Kressler, Millard-Stafford et al. 2011). The finding that supplementation can improve exercise performance is somewhat contradictory to the common belief that additional antioxidant intake does not significantly impact physical performance (Peternelj and Coombes 2011). Nonetheless, this assumption has been based on a large number of parallel placebo-controlled investigations using a relatively small subject number, which may skew the data and create a potential bias. In addition, many studies that have reported no benefit or even a reduction in performance have examined the efficacy of supplements such as vitamin C and / or vitamin E (Bryant, Ryder et al. 2003; Fry, Bloomer et al. 2006; Zoppi, Hohl et al. 2006; Knechtle, Knechtle et al. 2008; Teixeira, Valente et al. 2009). Previous research has suggested that performance

benefits will only be observed with AOX mixture supplementation if the participants were previously deficient in these vitamins and minerals (Watson, Callister et al. 2005). Furthermore, scant data is provided in a number of the placebo-controlled investigations regarding the physical training completed and nutritional intake during prolonged supplementation periods, which may also distort the results. Collectively, these findings suggest that acute to short-term supplementation protocols with antioxidants such as NAC, polyphenols or coenzyme Q_{10} improve performance. However, further well-controlled investigations are required to firmly establish the ergogenic effects of antioxidant compounds in human subjects.

The current analysis identified NAC as the supplement which had the most beneficial effect on performance. N-acetylcysteine is a thiol containing compound which acts to minimise the oxidative insult through its actions as a cysteine donor in the maintenance of glutathione homeostasis and via direct scavenging of ROS (Cotgreave 1997). Initial investigations that reported the ability of NAC to inhibit skeletal muscle fatigue were conducted using an intravenous infusion (Reid, Stokic et al. 1994; Travaline, Sudarshan et al. 1997). More recently, oral supplementation with NAC has produced similar performance benefits (Matuszczak, Farid et al. 2005; Kelly, Wicker et al. 2009; Corn and Barstow 2011). To determine the relative effects for oral *vs.* infusion supplementation protocols with NAC, the mean effect size of each protocol was calculated. When separated, a moderate effect size was demonstrated for both oral NAC supplementation (g = 1.03; CI 0.25, 1.79) and NAC intravenous infusion (g = 0.83; CI 0.28, 1.38). Therefore, performance enhancements can be achieved using either oral or infused NAC supplementation protocols. This suggests that NAC can be used to improve performance in both a laboratory setting and sporting environment.

The effect size of the supplementation loading periods in the current analysis closely corresponded to the type of antioxidant compound examined. For instance, acute supplementation protocols were shown to have the greatest effect on performance and were predominately used in the NAC investigations, which also had the largest effect size. Similarly, short-term loading periods were revealed as the next most effective strategy and were mostly used in studies which examined the ergogenic effects of polyphenols. Both prolonged loading periods and AOX mixtures were also observed to have the lowest effect

on performance. These findings suggest that the type of antioxidant compound consumed has a greater effect on performance as opposed to the loading period.

It is difficult to draw a definitive conclusion on the larger effect of antioxidant supplementation on resistance based performance tasks compared to aerobic capacity due to the relatively few number of prior investigations. A possible underlying mechanism for this observation may be that type II muscle fibres utilised during resistance exercise have a reduced concentration of endogenous antioxidant enzymes and are more vulnerable to ROS damage (Ji, Fu et al. 1992). Moreover, factors such as substrate availability and oxygen kinetics may have a greater impact on the development of fatigue during whole body aerobic exercise rather than an overproduction of ROS. Further research is necessary to assess the most appropriate type of antioxidant compound and supplementation protocol to enhance both resistance and aerobic capacity using performance tests, which closely reflect the demands of competition (Powers, Smuder et al. 2010).

Summary

The systematic review revealed that antioxidant compounds could have a small to moderate effect on performance. Acute supplementation of either oral or infused NAC was demonstrated to elicit the greatest performance improvements. However, further examination using placebo-controlled crossover experimental research designs are still required to establish the ergogenic effect and appropriate dosage of antioxidant supplementation in a sports setting. A large proportion of previous antioxidant supplementation studies have used laboratory based experimental designs to investigate performance changes associated with antioxidant supplementation, which may not be directly applicable to athletes in a competitive environment. Furthermore, future research should be conducted in either physically active or well-trained individuals as artificially higher effect sizes may occur if the study is conducted using untrained subjects. The length of the supplement loading period also deserves close consideration, as prolonged antioxidant supplementation may interfere with redox signalling cascades and blunt training-induced adaptations (Ristow, Zarse et al. 2009).

Interplay between Inflammatory, Hormonal and Oxidative Response during Exercise

Oxidants and Exercise-Induced Inflammation

The mechanical and metabolic stress of exercise can result in damage to the structural integrity of myofibres and cause temporary reductions in contractile function. Following exercise-induced injury, an inflammatory cascade is activated via the up-regulation of pro-inflammatory cytokines, chemokines and stress hormones that act as chemical messengers to modulate the repair and regenerative processes (Pedersen and Hoffman-Goetz 2000). Inflammation is characterised by the mobilisation and infiltration of phagocytic cells, which release proteolytic enzymes and ROS to remove necrotic tissue and cellular debris (Pyne, Baker et al. 1996). This response is associated with an increased membrane permeability and subsequent leakage of the intracellular enzymes CK and lactate dehydrogenase (LDH) from muscle tissue, combined with the hepatic release of acute-phase proteins such as CRP, transferrin and α-macroglobulin (Pedersen and Hoffman-Goetz 2000). Once necrotic tissue is removed, satellite cells proliferate to regenerate skeletal muscle tissue through the regulation of transcription factors (Podhorska-Okolow, Sandri et al. 1998). It is through this process that the exercise-induced inflammation reaction plays an important role in cellular remodelling and enhancement of the proteolytic pathways to promote hypertrophic adaptations (Stupka, Tarnopolsky et al. 2001).

The oxidant system is closely involved in the inflammatory process and contributes to the aetiology of exercise-induced muscle damage through a number of mechanisms (Hellsten, Frandsen et al. 1997; Vider, Lehtmaa et al. 2001; Tidball 2005). For example, during skeletal muscle contraction, the ROS released through mitochondrial respiration can cause direct oxidative damage to protein structure (Yu 1994). As a result, oxidised proteins often become functionally inactive and are highly susceptible to proteolytic degradation, rendering them unable to perform normal cellular functions (Dean, Fu et al. 1997). The damaged proteins are then removed by the activation of the acute phase inflammatory response. At the site of the injury, circulating neutrophils generate additional ROS via an 'oxidative burst' (Babior 1999). This reaction is catalysed by the enzyme nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase which reduces extracellular oxygen to form the superoxide anion (Babior 1999). Superoxide can then react to form other ROS including hydrogen peroxide, hypochlorous acid, and the hydroxyl radical (Droge 2002).

Together, these unstable oxygen-derived species destroy the apoptotic and necrotic cells. These powerful oxidants may also cause damage to the neutrophil itself and healthy cells near the site of inflammation (Babior 1999). Indeed, for up to 48 h post the initial exercise bout, further ROS generation via NADPH oxidase activity and increased xanthine oxidase activation during ischemia reperfusion can continue to cause injury and inflammation reactions in the skeletal muscle tissue (Brickson, Hollander et al. 2001; Dong, Chen et al. 2011).   However, in properly periodised training programs, the exercise-induced inflammatory response is well controlled and acts to facilitate the repair and restoration of contractile function.

Increased ROS release can also mediate signalling pathways to modify gene expression of transcription factors involved in the inflammatory cascade. The redox sensitive transcription factor, NF-κB has been extensively researched in its role as a central mediator of the stress-response to exercise (Kramer and Goodyear 2007). Activation of NF-κB has been shown to occur via a number of exercise-induced stimuli, including ROS, cytokines, elevated intracellular calcium, thyroid hormones and the MAPK pathway (Ji, Gomez-Cabrera et al. 2004). NF-κB is located in the cell cytoplasm bound to IκB inhibitor proteins (IκB). When NF-κB is activated, the IκB is phosphorylated, allowing the NF-κB to rapidly dissociate, translocate into the nucleus and bind to the DNA sequence of target genes (Janssen-Heininger, Poynter et al. 2000). It is in this manner that NF-κB can trigger the release of pro-inflammatory mediators including chemokines, cytokines, acute-phase proteins and adhesion molecules to facilitate the regenerative responses in damaged skeletal muscle (Aoi, Naito et al. 2004). The NF-κB pathway also promotes cell protection via the transduction of antioxidant enzymes (i.e. superoxide dismutase) within skeletal muscle (Gomez-Cabrera, Borras et al. 2005). Combined, these results demonstrate that the transient activation of the NF-κB pathway promotes adaptive responses to increase the functional capacity of skeletal tissue. Although, when NF-κB is chronically activated it can cause dysregulation within the oxidative and metabolic systems leading to unwanted skeletal muscle atrophy and insulin resistance (Kramer and Goodyear 2007). This sustained activation of NF-κB may occur due to exercise-induced oxidative perturbation of the redox balance during periods of intensified physical training (Droge 2002).

Eccentric, prolonged, high-intensity, strenuous or unaccustomed bouts of exercise have been associated with substantial increases in contractile-induced damage and inflammation

reactions (Pyne, Smith et al. 2000). Following these types of exercise, the functional performance capacity of skeletal muscle is likely to be reduced for 24 to 96 h post the initial injury (MacIntyre, Sorichter et al. 2001; Marcora and Bosio 2007; Ascensao, Rebelo et al. 2008). Whilst exercise-induced inflammation is a necessary precursor to muscle growth, the uncontrolled proliferation of inflammatory cells and oxidants can exacerbate muscle damage. Additionally, if the injury stimulus is repeated prior to restoration of muscle function, a state of low grade chronic inflammation may develop (Smith 2004). Dysregulation in the inflammatory system has been observed in athletes undergoing intense periods of physical training, as evidenced by excessive delayed-onset-muscle soreness, muscle stiffness, reduction in muscle strength, increased CK activity and impaired immune function (Urhausen, Gabriel et al. 1998; Hedelin, Kentta et al. 2000; Collins, Renault et al. 2003). To prevent the potential negative effects of inflammation, the efficacy of nutritional antioxidant interventions to minimise the exercise-induced oxidative damage has been widely investigated.

Effect of Antioxidant Supplementation on Inflammation

Excessive exercise-induced muscle damage and inflammation can cause significant reductions in athletic performance capacity (Twist and Eston 2005; Marcora and Bosio 2007) and lead to a reduced tolerance of demanding physical training loads (Smith 2004). The consumption of exogenous antioxidants supplements may decrease the oxidative insult and subsequent inflammation reaction to exercise (Diaz-Castro, Guisado et al. 2011). Studies have investigated the ability of antioxidant compounds to minimise the inflammatory response using an array of exercise modalities. Antioxidants have been reported to effectively reduce post-exercise markers of inflammation, such as CRP and minimise the up-regulation of pro-inflammatory cytokines (Phillips, Childs et al. 2003; Howatson, McHugh et al. 2010; Diaz-Castro, Guisado et al. 2011). Additionally, exogenous antioxidant supplementation can accelerate recovery of muscle function following damaging exercise (Gauche, Lepers et al. 2006; Bowtell, Sumners et al. 2011; Trombold, Reinfeld et al. 2011). It is therefore unsurprising that prolonged antioxidant supplementation periods have also been shown to reduce the cumulative inflammatory effects experienced by athletes during periods of high intensity training (Funes, Carrera-Quintanar et al. 2011; Nishizawa, Hara et al. 2011). Thus, it is evident that an additional antioxidant intake, whether causally or indirectly, can decrease the exercise-induced inflammatory response. Although, a reduction in inflammatory mediators with antioxidant supplementation has not been consistently

shown (Dawson, Henry et al. 2002; Nieman, Henson et al. 2002; Mastaloudis, Morrow et al. 2004; Konrad, Nieman et al. 2011).

The differing results from the studies that have examined the efficacy of antioxidants to combat exercise-induced inflammation may be due to a number of factors, including exercise type, antioxidant compound / dosage and the training status of the participants investigated. For instance, several studies which have shown no effect of antioxidant supplementation on the inflammation reaction used an exercise model that was extremely strenuous (i.e. ultra marathons, prolonged cycling) (Dawson, Henry et al. 2002; Nieman, Henson et al. 2002; Mastaloudis, Morrow et al. 2004; Konrad, Nieman et al. 2011) or utilised purposefully damaging eccentric exercise bouts (Thompson, Bailey et al. 2004). It is likely that when severe mechanical stress is placed on contractile fibres, damage and inflammation will occur irrespectively of any additional antioxidant protection. Supra-nutritional doses of antioxidants have also been demonstrated to exert a pro-oxidant effect, which can exacerbate muscle damage (Childs, Jacobs et al. 2001). In addition, prolonged antioxidant supplementation may impair redox signalling of endogenous antioxidant enzyme gene up-regulation and leave cells more susceptible to oxidative damage (Gomez-Cabrera, Borras et al. 2005). Another possible explanation is that an increased level of inflammation and muscle damage after acute bouts of exercise may be indicative of a greater amount of work completed with the support of antioxidant supplements. For example, when 12 recreationally trained males consumed the antioxidant, NAC, an increased level of muscle damage was associated with significant improvements in repeat sprint running performance (Cobley, McGlory et al. 2011). Due to this complex relationship between redox balance and the exercise-induced inflammatory response, it is difficult to establish guidelines for antioxidant supplementation in athletes.

Contention exists amongst sport and exercise researchers as to whether minimising oxidative stress and inflammation is beneficial or detrimental to physiological adaptation and athletic performance (Gomez-Cabrera, Domenech et al. 2008; Higashida, Kim et al. 2011). Inflammation is an important stage in the repair and regeneration of damaged skeletal muscle tissue (Tidball 2005). If the inflammatory response is restricted by antioxidant supplementation, recovery from exercise-induced skeletal muscle damage may be impaired, resulting in an increased oxidative stress response during subsequent bouts of exercise (Close, Ashton et al. 2006). Conversely, the consumption of additional exogenous

antioxidant supplements can decrease the exercise-induced oxidative insult and systemic-stress reaction during intensified training periods (Palazzetti, Rousseau et al. 2004). For instance, antioxidant supplementation has been associated with a reduced secretion of the stress hormone cortisol (Peters, Anderson et al. 2001; Davison, Gleeson et al. 2007; Teixeira, Valente et al. 2009). It is currently not clear whether this observed reduction in hormone release is due to a systemic reduction in exercise-stress, or a direct interaction between the redox, inflammatory and neuroendocrine systems.

Oxidants and the Neuroendocrine Response to Exercise

The neuroendocrine system plays an important role in maintaining homeostatic control during exercise bouts (Viru 1991). It is a complex and integrative system of communication between the central nervous system, endocrine glands, hormones and target cells (Hackney 2006). In response to an exercise stress, hormones are released via the hypothalamic-pituitary-adrenocortical axis and sympathetic-adrenal-medullary axis, that bind to specific receptors expressed on target cells to trigger a physiologic response (Hackney 2006). Levels of circulating hormones are rapidly elevated in response to an acute exercise bout and are influenced by a multitude of exercise related factors (i.e. exercise duration, intensity and mode) and non-exercise related factors (i.e. psychological stress, temperature, nutrition, hypoxia, hydration status and circadian rhythm) (Viru and Viru 2001). Adaptation and accommodation within the neuroendocrine stress response has been observed through an attenuation of hormone secretion (i.e. cortisol and catecholamines) during exercise at the same relative workload (Steinacker, Lormes et al. 2004). In general, exercise-induced increases in circulating hormones are transient and levels return to baseline or slightly below basal values at the cessation of physical activity (Hackney 2006). However, following extremely stressful exercise or during intensified periods of training, prolonged endocrine disturbances are likely to occur (Steinacker, Lormes et al. 2004). This sustained rise in circulating hormones may lead to an increased oxidant release and can negatively affect homeostasis via the promotion of a catabolic signalling environment.

Elevations in catecholamine (Podolin, Munger et al. 1991) and thyroid hormone (Ciloglu, Peker et al. 2005) occurs at the onset of exercise to assist in the regulation of metabolic, muscular and cardio-circulatory reactions. However, the exercise-induced and / or sustained elevations in these hormones during periods of chronic stress may also contribute to a greater amount of oxidative stress. Early research by Misra and Fridovich (1972)

demonstrated experimentally that the auto-oxidation of catecholamines is an additional source of the ROS, superoxide. The increase in circulating catecholamines during exercise can also bind to beta-adrenergic receptors, which elevate sympathetic activity and augment skeletal muscle oxidative metabolism (Mehta and Li 2001).   This increase in cellular respiration may result in a greater oxygen flow and ROS (superoxide) formation through the mitochondrial electron transport chain (Turrens 1997).   Likewise, elevations in thyroid hormones can promote a hyper metabolic state resulting in an increased oxygen flux and subsequent superoxide release from the mitochondria (Venditti and Meo 2006).   This additional release of ROS may then start a chain reaction in which superoxide is the propagating species resulting in oxidative cellular damage and the loss of cell function. Increases in oxidant production have also been linked to thyroid hormone-induced hyperplasia and hypertrophy of phagocytic cells, leading to increased superoxide formation via oxidative burst activity during inflammation reactions (Tapia, Fernandez et al. 2003). Furthermore, the thyroid hormones can induce NF-κB activation, which if sustained for prolonged periods, may cause cachexia and muscle wastage (Ho, Hirshman et al. 2005). These factors combined, suggest that exercise-induced activation of the neuroendocrine system can contribute to oxidative muscle injury (Asayama and Kato 1990).

The neuroendocrine and redox systems are both integral in coordinating the metabolic response to a stress stimulus.   A synergistic action occurs between exercise-induced increases in glucocorticoids (i.e. cortisol) and insulin hormones to facilitate glucose transport and promote lipolysis to meet the energy demands of physical activity (Sjostrand and Eriksson 2009).   Likewise, ROS signalling has also been demonstrated to, in part, mediate glucose uptake to the skeletal muscle during exercise (Balon and Yerneni 2001). Additionally, redox sensitive genes, PPARγ and MAPK initiate positive adaptive mechanisms to increase insulin sensitivity and promote cellular remodelling and regeneration (Ji 2008; Ristow, Zarse et al. 2009).   This metabolic up-regulation creates an anabolic environment, which is conducive to cellular growth and adaptation. However, prolonged perturbations in both the redox and allostatic balance during strenuous training periods can disrupt the normal regulatory effects of the neuroendocrine system (Sjostrand and Eriksson 2009; Costantini, Marasco et al. 2011). A pro-oxidant shift in redox sensitive signalling pathways may also impair glucose transport and increase insulin resistance within skeletal muscle due to altered patterns of gene expression within pro-inflammatory transcription factors (i.e. NF-κB) (Balon and Yerneni 2001; Brownlee 2005). This resultant increase in inflammation may

promote further oxidative damage and impair protein translocation and synthesis leading to skeletal muscle atrophy (Powers, Duarte et al. 2010). Furthermore, it has been demonstrated that damaged myofibres have a reduced glycogen resynthesis rate, which may also contribute to metabolic dysregulation and a reduced performance capacity during strenuous training periods (Asp, Daugaard et al. 1998). These findings highlight that exercise performance and physiological adaptation are reliant on the collective inputs from the oxidative, inflammatory and hormonal systems.

Hormesis, Exercise and the Inflammatory, Neuroendocrine and Oxidative Systems

Significant interplay exists between the oxidative, inflammatory and neuroendocrine reaction to an exercise stimuli. Physiological changes in response to acute bouts of exercise and training-induced adaptations are reliant on the proper functioning and cohesion amongst the respective systems. At the onset of exercise, rapid alterations in the intra-cellular redox milieu occur, which acts to regulate biological responses and mediate cellular adaptation via the induction of gene transduction pathways (Powers, Duarte et al. 2010). It is through these adaptive processes that the body is able to tolerate progressive increases in training load. However, an over or under production of ROS may cause interference with redox sensitive signalling cascades, resulting in a state of chronic inflammation and dysregulation within the neuroendocrine system (Radak, Chung et al. 2005). The theory of hormesis explains the apparent dichotomy in the occurrence of both positive and negative physiological responses in reaction to perturbations within the redox balance (Radak, Chung et al. 2008). A hormetic effect is characterised by a bidirectional dose-response relationship, whereby at a low dose, potentially toxic substances stimulate beneficial effects, yet a high dose of the same substance is inhibitory (Calabrese and Baldwin 2003). Indeed, the principle of hormesis can also be extrapolated to encompass a variety of the observed exercise-induced pathophysiological reactions to physical training and the resultant impact on performance capacity (Figure 2.3).
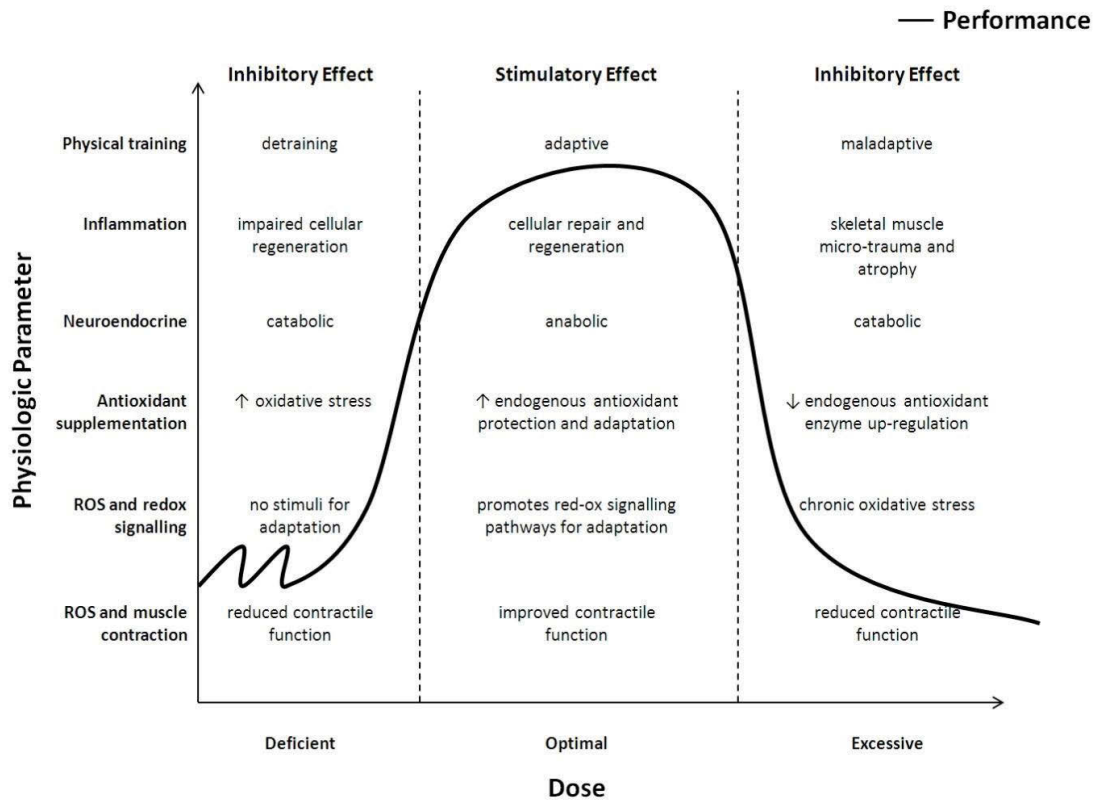
Figure 2.3: The hormetic effect of exercise parameters on performance. Modified from Droge (2002).

The concentration of ROS has been shown to exert a hormetic response during skeletal muscle contraction. For instance, a low myofibril concentration of ROS can enhance calcium release from the sarcoplasmic reticulum and increases force production (Reid 2001). However, high concentrations of ROS have been linked to decreases in contractile force due to an inhibition of calcium sensitivity (Andrade, Reid et al. 2001), dysregulation of muscle Na^+,K^+-pump activity (McKenna, Medved et al. 2006) and reduced mitochondrial integrity (Di Meo and Venditti 2001). Hormesis is also evident in the inflammatory response to exercise. Whilst the inflammatory reaction is an important step in the regeneration of damaged muscle fibres, chronic activation of inflammatory mediators may lead to immuno-suppression and skeletal muscle atrophy (Tiidus 1998; Smith 2004). Similarly, exercise-induced secretion of glucocorticoid hormones can exert both a stimulatory anabolic effect on glucose metabolism and a catabolic effect by the inhibition of protein synthesis (Steinacker, Lormes et al. 2004). In essence, exercise is also an example of hormesis. Habitual physical activity simulates up-regulation of the antioxidant defence system and greatly benefits health and well-being (Gomez-Cabrera, Domenech et al. 2008). In contrast, excessive

exercise can lead to a sustained increase in oxidative stress resulting in a greater susceptibility to illness and an impaired performance capacity (Tiidus 1998). These examples highlight the importance of finding a balance between physical training loads and recovery periods to provide the optimal hormetic dose of exercise stimuli, which improves performance in a practical sports setting.

Limitations and Directions for Future Research

Despite considerable research investigating the stress-response and adaptation to exercise, the physical training dose required to produce an optimal performance for each individual remains unclear. A major confounding factor is the complex nature of exercise adaptation. Most bioactive substances can induce both a positive and negative physiological response. It is therefore important to investigate the respective changes in a variety of biological systems to ensure an accurate and holistic assessment of training-induced perturbation. To date, comparatively few studies have examined the synergistic response within oxidative, inflammatory and neuroendocrine systems to exercise stress in well-trained athletes (Suzuki, Totsuka et al. 1999; Palazzetti, Richard et al. 2003). In addition, a large number of studies have utilised animal models to assess the process of physiological adaptation during relatively short periods (1–3 months), which may have limited relevance to an athletic population (Gomez-Cabrera, Borras et al. 2005; Higashida, Kim et al. 2011). These animal-based experiments have provided valuable insight and an increased understanding of adaptation at a transcriptional level. However, an up-regulation in gene expression may not directly transfer to an increase in post-translational protein synthesis and / or performance improvements (Powers, Duarte et al. 2010). Further research is required to gain a more in depth understanding of the synergistic responses within the oxidative, inflammatory and neuroendocrine systems to modulate exercise-induced physiological adaptation.

Summary and Conclusions

Exercise triggers a systemic stress response and up-regulation of biological systems to meet the additional work demands. This disruption in homeostasis activates a series of signalling cascades designed to restore balance and increase tolerance to subsequent bouts of exercise. It is through this process that physical training improves athletic performance capacity. Indeed, exercise-induced changes within the oxidative, inflammatory and

neuroendocrine systems are heavily involved in the adaptive process to a training stimulus. The proper functioning of these systems may be impaired during acute bouts of strenuous exercise and chronic dysregulation has been observed in excessively fatigued athletes. It has been suggested that exogenous supplementation of antioxidants may reduce the stress response to exercise and assist athletes to cope with increased training loads. Short-term supplementation with the antioxidants, NAC and quercetin, has also been demonstrated to exert an ergogenic effect on performance. However, a reduction in exercise-stress may also attenuate redox mediated adaptive process. Further examination is therefore required to appropriately titrate the additional antioxidant requirements for individuals during periods of increased training stress, which will promote redox regulated gene transcription and enhance athletic performance.

CHAPTER THREE

Antioxidant intake of well-trained athletes during intensified physical training

Slattery, K.M., Wallace L.K. and Coutts, A.J. (*in press*). Antioxidant intake of well-trained athletes during intensified physical training. *J Sports Med Phys Fit*.

Abstract

The nutritional antioxidant intake of well-trained athletes during a block of intensive exercise and the associated changes in well-being was examined. The dietary intake (24 h food recall) and Daily Analysis of Demands for Athletes (DALDA) questionnaire of 23 well-trained athletes was monitored during a 4 d intensive physical training period. The results showed the mean antioxidant vitamin intake for well-trained athletes was above the Australian RDI ($P < 0.01$). Individual analysis revealed that not all participants consumed sufficient amounts of vitamin A (29%) and / or vitamin E (25%). No difference was observed in training load or 'worse than normal' DALDA responses between the vitamin sufficient and vitamin deficient athletes. It was concluded that well-trained athletes have an adequate dietary intake to allow for optimal physical training and should not require exogenous antioxidant supplementation.

Key words: vitamin C, vitamin A, vitamin E, training load

Introduction

Many well-trained athletes habitually consume exogenous antioxidant supplements (Dascombe, Karunaratna et al. 2010). It has been suggested that athletes require an increased antioxidant intake due to a greater exposure to exercise-induced oxidative stress during physical training (Rousseau, Hininger et al. 2004). Previous dietary analyses on elite French (Rousseau, Hininger et al. 2004), Brazilian (Paschoal and Amancio 2004) and Greek (Farajian, Kavouras et al. 2004) athletes, have revealed an inadequate nutritional consumption of antioxidants from whole foods. Specifically, 81% of French, 71% of Brazilian and 93% of Greek athletes within the investigations were observed to have a deficient intake of at least one important antioxidant vitamin. This insufficiency in antioxidant consumption may reduce an athlete's ability to tolerate oxidative stress and increase susceptibility to muscle damage, illness and self-reported fatigue (Palazzetti, Rousseau et al. 2004; Watson, MacDonald-Wicks et al. 2005). For instance, a greater amount of exercise-induced oxidative damage was incurred when 17 trained athletes consumed a restricted antioxidant diet for 2 weeks, compared to a diet containing at least the Australian recommended daily intake of vitamin A and C (Watson, Callister et al. 2005). The consumption of antioxidant supplements may therefore be beneficial to reduce the exercise-increased oxidative response, minimise muscle damage and facilitate recovery (Palazzetti, Rousseau et al. 2004).

Additional antioxidant protection via supplementation has been shown to reduce post-exercise markers of oxidative damage and inflammation (Di Giacomo, Acquaviva et al. 2009; Arent, Pellegrino et al. 2010; Howatson, McHugh et al. 2010). This decrease in muscle disruption may facilitate the post-exercise recovery process and improve athlete tolerance to intensified training demands. Antioxidant supplementation may also help reduce training-induced stress-reaction symptoms (i.e. muscle soreness, unexplained aches, swelling, general weakness). However, the excessive consumption of antioxidants via supra-nutritional doses can also have a negative effect on athletic performance. Research has identified that exogenous antioxidants can blunt transcription of antioxidant enzyme gene expression and attenuate other regulatory pathways involved in exercise adaptation (Ristow, Zarse et al. 2009). Moreover, select studies have reported that antioxidant supplementation provides no additional protection against oxidative damage and inflammation (Davison and Gleeseon 2005; Skarpanska-Stejnborn, Pilaczynska-Szczesniak et al. 2009; Teixeira, Valente et al. 2009), or may even exacerbate oxidative damage and inflammation during strenuous exercise

(Close, Ashton et al. 2006). To date, there is limited information on the relationship between antioxidant status and an athlete's well-being.

Collectively, these findings suggest that athletes may require additional antioxidant support during periods of intensified physical training or when dietary intake is inadequate (Rousseau, Hininger et al. 2004; Burke L 2005). Due to the conflicting results on the benefits and / or detriments of added antioxidants, it is important to establish the dietary antioxidant intake of well-trained athletes prior to recommending supplementation. Therefore, the purpose of the current investigation was to determine the nutritional antioxidant intake of well-trained athletes during a block of intensive exercise and the associated changes in well-being.

Methods

Participants

Twenty three well-trained athletes volunteered to participate in this study. Participants were either at a regional or national level in their chosen sport. The cohort consisted of 6 (4 male, 2 female) nationally ranked swimmers (age 19.8 ± 3.3 y, height 177.5 ± 10.1 cm, body mass 74.7 ± 12.2 kg), 7 male team sport athletes (age 20.3 ± 2.4 y, height 184.1 ± 3.3 cm, body mass 80.5 ± 8.1 kg) and 10 male triathletes (age 23.6 ± 3.2 y, height 179.8 ± 4.4 cm, body mass 70.5 ± 7.2 kg) who compete at a regional to international standard. All athletes gave informed consent and were able to withdraw from the study at any time. The study was approved by the University of Technology, Sydney Human Ethics Committee and complied with the Helsinki Declaration.

Experimental design

Dietary intake (24 h food recall) was recorded on a daily basis during a 4 d intensified training period. Participants were given detailed verbal and written instructions on how to accurately record food intake using household measures and were asked to maintain their usual diet during the testing period. Participants were not taking additional antioxidant supplements. Food records were analysed using the Foodworks program (version 6, 2009, Xyris Software, Australia). Following the dietary analysis, the 4 d mean nutritional intake of each athlete was compared to the Australian recommended daily intake (RDI) of vitamin C (45 mg), vitamin E (female = 7 mg, male = 10 mg) and total vitamin A (retinol equivalents and β-carotene) (900 μg) for Australians(National Health and Medical Research Council 2005)

(NHMRC 1991). Training load (AU) was assessed using the session-RPE method (Foster, Florhaug et al. 2001), which is calculated as the product of training duration (min) and the mean training intensity (rating of perceived exertion CR-10) (Borg, Hassmen et al. 1985). A period of intensified training was defined as a minimum 25% increase in training load from the preceding week. All training sessions were supervised by the athlete's respective coach, or the principal investigator. Participants also completed the Daily Analyses of Life Demands for Athletes (DALDA) questionnaire, at the same time each day to assess general stress levels and stress-reaction symptoms (Rushall 1990).

Statistical analyses

Statistica 8.0 software (StatSoft Inc. Tulsa, USA) was used for all calculations. A paired samples t-test was used to determine differences in antioxidant intake compared to the Australian RDI. For closer analysis, the data was divided into two groups, those that fulfilled the RDI for all vitamins (antioxidant sufficient) and those that did not (antioxidant deficient). An independent t-test was used to determine the difference between training load and 'worse than normal' DALDA responses within the antioxidant sufficient and deficient groups. Cohen's d were calculated and interpreted as trivial (0.1 – 0.19), small (0.2 – 0.59), moderate (0.6 – 1.19), large (1.2 – 3.9) and extremely large (> 4.0) (Cohen 1988). Spearman's Rank correlation analysis was performed to determine relationships between the measured variables. Statistical significance was accepted at $P < 0.05$. Data are presented as mean ± SD.

Results

The dietary analysis of the participants is shown in Table 3.1. Group results showed a significantly greater intake of vitamin A (d = 0.76, P = 0.013), vitamin C (d = 1.39, P = 0.001) and vitamin E (d = 0.86, P = 0.001) was consumed compared to the Australian RDI. Individual analysis revealed that 29% and 25% of participants consumed below the RDI for vitamin A and vitamin E, respectively (Figure 3.1). Training load (AU) and the number of 'worse than normal' DALDA responses for each participant are shown in Figure 3.2. No significant main effects and small effect sizes were observed between antioxidant deficiency and training load (d = 0.41, P = 0.27) or 'worse than normal' DALDA responses (d = 0.30, P = 0.606). No correlation was observed between antioxidant intake, 'worse than normal' DALDA responses and training load.

Table 3.1: Mean nutritional intake for well-trained athletes during a 4 d intensive training period (NHMRC 1991; Burke L 2005).

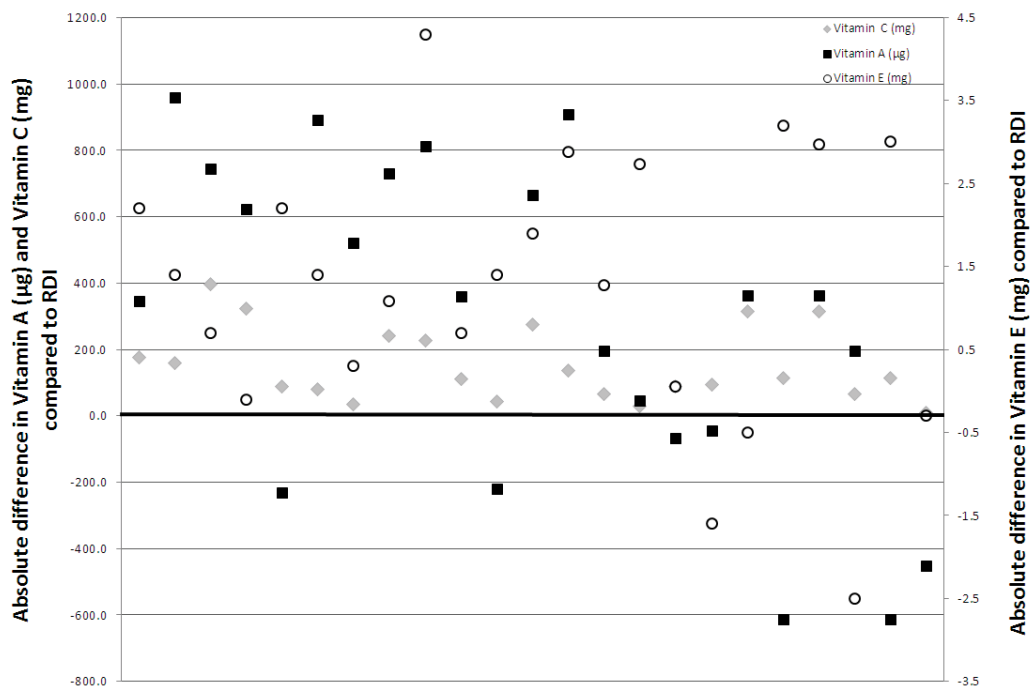| Nutrients	Mean ± SD	Range	Recommended Daily Intake
Energy Intake (kJ)	13052 ± 3321	7939 – 19277	
Carbohydrate (g/kg)	5.5 ± 1.7	2.5 – 9.8	7 - 10
Carbohydrate (g)	407 ± 132	207 – 732	
Protein (g/kg)	2.1 ± 0.6	1.0 – 3.1	1.4 - 1.6
Protein (g)	160 ± 54	74 – 272	
Fat (g)	85 ± 30	34 – 160	
Vitamin A (µg)	1243 ± 605	287 – 2929	900
Vitamin C (mg)	197 ± 110	55 – 441	45
Vitamin E (mg)	10.9 ± 1.7	7.5 – 14.3	7 - 10

Figure 3.1: Actual mean intake of vitamin A, vitamin C and vitamin E during 4 d intensified physical training compared to the Australian RDI.
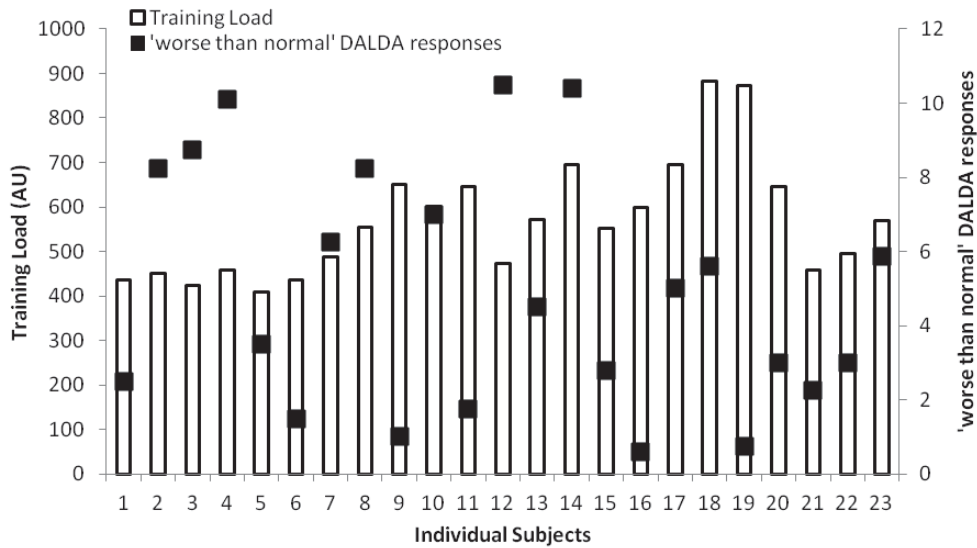
Figure 3.2: Mean 4 d training load and corresponding 'worse than normal' DALDA score for each participant.

Discussion

The results of the present investigation showed that collectively, well-trained athletes consumed above the RDI for common antioxidant vitamins A, C and E. Similar high levels of antioxidant intake have been reported in well-trained male endurance Australian athletes who consumed 139 ± 14 mg of vitamin C and 1347 ± 157 µg of vitamin A equivalents in their habitual diet (Watson, Callister et al. 2005). The observed increase in vitamin consumption may be a by-product of the relatively greater food intake required by athletes to complete large training loads. In accordance with the higher carbohydrate and protein recommendations for athletes to meet the additional energy demands of physical training (Burke L 2005), it has been suggested that the antioxidant RDI for athletes should also be increased (i.e. from 45 to 200 mg/d of vitamin C) (Rousseau, Hininger et al. 2004; Burke L 2005). Indeed, the vitamin C intake of 39% of the athletes in the current investigation was above the higher athlete RDI of 200 mg·d^{-1}. Most athletes are able to consume above the RDI for vitamin A, C and E by consuming a variety of high-antioxidant foods (Margaritis and Rousseau 2008). It is therefore likely that this higher antioxidant intake can be achieved without exogenous antioxidant supplementation.

The findings of the present study are in contrast to previous investigations, which have reported inadequate dietary intakes of antioxidant vitamins in well-trained athletes

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(Farajian, Kavouras et al. 2004; Paschoal and Amancio 2004; Rousseau, Hininger et al. 2004). These differing results occurred despite using a similar method (4 d food records) to assess nutritional intake. Notably, not all athletes in the current investigation fulfilled the daily requirements for vitamin A and vitamin E. Although, this insufficient antioxidant intake did not affect the stress-reaction symptoms reported in the DALDA. A 4 d intensive training period may be insufficient to observe the typical dose-response relationship between increased physical training load and an exercise-stress state. Nonetheless, the adequate matching of training load and antioxidant intake plays an important role in the maintenance of an athlete's health and well-being. Several investigations have demonstrated that athletes undergoing intensive training periods without adequate antioxidant support incur a greater level of oxidative stress (Schippinger, Wonisch et al. 2002; Palazzetti, Rousseau et al. 2004; Watson, Callister et al. 2005). The consumption of antioxidant supplements during strenuous training periods has also been shown have a protective effect on muscle integrity (Itoh, Ohkuwa et al. 2000; Funes, Carrera-Quintanar et al. 2011). These results show that inadequate antioxidant protection during strenuous exercise can impair an athlete's tolerance to physical training loads. It is therefore important to identify athletes with a low dietary antioxidant intake, as they may have an increased susceptibility to excessive exercise-induced oxidative damage.

The antioxidant defence system reacts in a synergistic manner to control increases in oxidant production (Halliwell, Aeschbach et al. 1995). Each antioxidant compound has a specific function and role to perform. However, dietary analysis of antioxidant vitamins and minerals, according to Australian food tables, is currently limited to vitamin C, vitamin E and total vitamin A (retinol equivalents and β-carotene). Athletes may consume sufficient amounts of vitamin A, C and E, yet still be susceptible to exercise-induced oxidative insult. The inclusion of other antioxidant compounds such as polyphenols (i.e. quercetin, flavanoids), glutathione, coenzyme Q_{10}, lycopenes and minerals (i.e. selenium) would provide a clearer picture of total antioxidant intake. In particular, the dietary intake of polyphenols is of interest. Recent research has identified that quercetin supplementation has an ~3% ergogenic effect on endurance performance (Kressler, Millard-Stafford et al. 2011) and can facilitate the recovery process following exercise-induced muscle damage (Trombold, Reinfeld et al. 2011).

Despite numerous investigations, there is no clear consensus on the benefit of antioxidant supplementation for athletes (Williams, Strobel et al. 2006). Whilst antioxidant supplements have been demonstrated to successfully reduce exercise-induced oxidative damage (Di Giacomo, Acquaviva et al. 2009; Howatson, McHugh et al. 2010), blunt inflammatory responses (Howatson, McHugh et al. 2010) and attenuate stress hormone release (Davison, Gleeson et al. 2007), these results have not been consistently shown (Davison and Gleeseon 2005; Teixeira, Valente et al. 2009). There is also strong evidence to suggest that large doses of exogenous antioxidant supplementation can be detrimental to physiological adaption and delay the process of recovery (Peternelj and Coombes 2011). Further research is still required to establish the antioxidant requirements of well-trained athletes whereby health and well-being is maintained without causing adverse effects on performance or recovery. The findings of the present study suggest that athletes who consume a well-balanced diet were able to tolerate increases in training load and should not require additional vitamin A, C, or E supplementation. It is therefore important to assess the dietary intake of athletes prior to recommending antioxidant supplementation.

CHAPTER FOUR

Effect of training load on simulated team sport match performance

Slattery, K.M., Wallace, L.K., Bentley, D.J. and Coutts, A.J. (*in press*). Effect of training load on simulated team sport match performance. *Appl Phys Nut Met*.

Abstract

This study examined the effect of training load on running performance and plasma markers of anaerobic metabolism, muscle damage and inflammation during a simulated team sport match performance using a cross-over experimental design. Seven team sport athletes ($\dot{V}O_2$max 47.6 ± 4.2 mL·kg^{-1}·min^{-1}) completed a 60 min simulated team sport match before and after either 4 days of HIGH or LOW training loads. Venous blood samples were taken pre-match, immediately post-match and 2 h post-match for interlukin-6, monocyte chemoattractant protein-1 (MCP-1), creatine kinase (CK), lactate dehydrogenase, C-reactive protein, xanthine oxidase (XO) and hypoxanthine. Following the HIGH training load, sprint velocity decreased ($P < 0.001$) and total distance covered was reduced (HIGH 5495 ± 670 m, LOW 5608 ± 674 m, $P = 0.02$) in the match simulation compared to following the LOW training condition. Decreased performance capacity was accompanied by a significant increase in serum CK concentration (HIGH 290 ± 62, LOW 199 ± 33 U·L^{-1}, $P = 0.005$). The HIGH training also resulted in a decreased post-match hypoxanthine and MCP-1 and an increase in XO concentration 2 h post-match. Four days of increased training load reduced running performance during the match simulation and altered the metabolic and inflammatory response to high-intensity intermittent exercise.

Key Words: hypoxanthine, xanthine oxidase, fatigue, muscle damage, interlukin-6, monocyte chemoattractant protein-1

Introduction

Xanthine oxidase (XO) mediated free radical production has been associated with post-exercise muscle damage and inflammation (Hellsten, Frandsen et al. 1997; Vina, Gimeno et al. 2000). Research has demonstrated significant increases in XO activity following acute bouts of eccentric (Hellsten, Frandsen et al. 1997), anaerobic (Volek, Kraemer et al. 2002) and exhaustive exercise (Radak, Asano et al. 1995; Rasanen, Wiitanen et al. 1996). Whilst it has recently been established that XO mediated increases in reactive oxygen species may improve skeletal muscle force generation (Gomez-Cabrera, Close et al. 2010), greatly elevated XO production following high-intensity activity may contribute to the damage of fast twitch type II fibres which have an increased susceptibility to ischemia reperfusion injury (Bushell, Klenerman et al. 1996) and oxidative damage (Alessio 1993). Damage to these fibres can cause prolonged impairment of muscle function and reduced glycogen resynthesis rate resulting in a decreased recovery in performance capacity in the days following the initial exercise bout (Asp, Daugaard et al. 1998; Twist and Eston 2005). Twist and Eston (2005) have shown that peak power output and 10-m sprint performance were reduced in team sport athletes for up to 48 h following a bout of muscle damaging exercise. Similarly, Ascensao et al. (2008) reported that a soccer match induces increases in plasma markers of oxidative stress and muscle damage with a concomitant decrease in isokinetic knee joint peak torque and an ~5% reduction in 20 m sprint performance for 72 h. To date however, no studies have investigated the link between intensified training between-matches on muscle damage and inflammatory stress on the hypoxanthine / xanthine oxidase pathway on running performance in team sport athletes.

Reductions in performance capacity following exercise-induced muscle damage has practical implications for individuals undertaking prolonged intermittent high-intensity sports where repeated sprint bouts, rapid changes of direction, accelerations and decelerations are routinely performed (Ascensao, Rebelo et al. 2008). Muscle damage, oxidative stress and inflammation incurred during team sport matches, is likely to induce reductions in muscle strength and power, which may be prolonged if sufficient recovery periods are not provided (Kingsley, Wadsworth et al. 2005; Ispirlidis, Fatouros et al. 2008). Most previous research conducted on the time-course recovery of exercise-induced muscle damage following intermittent team sports have utilised experimental protocols whereby the subjects were instructed to rest for the 72–144 h following the matches (Ascensao, Rebelo et al. 2008; Ispirlidis, Fatouros et al. 2008). However, in practice, team sport athletes

continue training between each match throughout the competitive season, and therefore previous research models may not reflect the demands of team sport athletes. Additionally, no previous study has examined the impact of influencing recovery from team sport matches on subsequent performance.

Therefore, the aim of this research was to examine the effect of prior training on sprint ability and alterations in plasma markers of anaerobic metabolism during a simulated team sport match performance. It was anticipated that a higher training load would induce a significantly greater level of exercise-induced muscle damage, oxidative stress and inflammation, which may then result in a reduced performance capacity due to an inability to sufficiently activate the anaerobic metabolism.

Methods

Participants

Seven trained male team sport athletes (age 20.3 ± 2.4 y, body mass 80.5 ± 8.1 kg, $\dot{V}O_2$max 47.6 ± 4.2 ml·kg·min^{-1}) volunteered to take part in the investigation. Prior to the study, subjects were informed of the testing requirements and potential risks involved in the study and gave written consent. The subjects participated at a club to regional level in their chosen sport (4 rugby union, 3 football players). The subjects typically completed four training sessions per week for their chosen sport. The study was approved by the university's Human Ethics Committee and was conducted in accordance with the Helsinki Declaration.

Experimental design

A randomly assigned, crossover experimental design was used to determine the effects of prior training load on intermittent running performance and blood markers of anaerobic metabolism and inflammation. Pre-testing and familiarisation on a non-motorised treadmill (NMT) (Woodway Force 3.0, Waukesha, WI) was completed 2 wk prior to the testing period. Subjects then undertook two 6 d testing periods separated by a 1 wk washout period. A 60 min team sport match simulation protocol on a NMT was completed prior to and following 4 d of either HIGH or LOW training loads (see Figure 4.1). To ensure a similar training load preceding each testing week, the subjects were required to complete a training diary for the week prior to the first experimental period. The subjects then repeated the same training during the washout period between the two testing weeks.
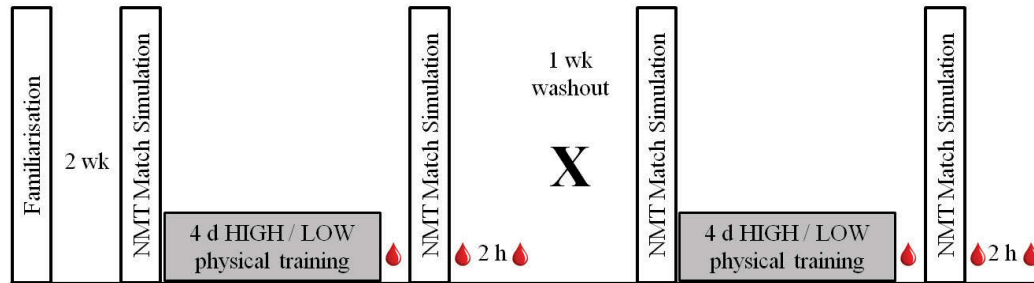
Figure 4.1: Experimental design. Non-motorised treadmill (NMT), 🔴 venous blood sample.

Team sport match simulation

The primary performance measure for this study was a team sport match simulation on a NMT. The exact treadmill system used in this investigation has been previously described in detail (Sirotic and Coutts 2007). Treadmill running belt velocity, distance, and horizontal forces were collected at a sampling rate of 10 Hz using the XPV7 PCB interface (Fitness Technology, Adelaide, Australia) and analysed using the Force 3.0 Software (Innervations, Joondalup, Australia). The reliability of the treadmill system in our laboratory has previously been established (TEM = 1.9%; ICC = 0.93) (Sirotic and Coutts 2007).

The 60 min NMT protocol was based on time-motion analyses to closely replicate the specific running movement demands which occur during team sport match-play (Sirotic and Coutts 2007). Six running speeds, based on a percentage of each subject's individual peak sprinting speed were used in the protocol; stand still (0%), walk (20%), jog (35%), run (45%), fast run (65%) and sprint (100% or maximal effort). Customised software was used to generate audio and visual signals for change of speed and real-time velocity feedback (to assist the subjects meet their individual target speeds).

Subjects repeated the same 15 min protocol, four successive times with a 10 min rest period between the second and third repetition to mimic a half-time break. A total of nine maximal sprints were incorporated into each 15 min period, including a 3 x 3 s repeat sprint ability test placed at the end of the protocol. Capilliarised blood lactate samples (Lactate Scout, Senslab, Leizpig, Germany) and rating of perceived exertion (category ratio 1-10) (Borg, Hassmen et al. 1985) were taken after 6 min of exercise in each period and at the immediate conclusion of the second and fourth period.

Pre-testing protocols

Subjects were required to attend two pre-testing laboratory sessions, at least 48 h apart. During pre-testing session 1, familiarisation with the NMT protocol, maximal oxygen uptake ($\dot{V}O_2$max) and three repetition maximum (3RM) bench press were completed. A second familiarisation and peak speed assessment on the NMT and the YoYo Intermittent recovery test (level 1) were completed during pre-testing session 2. The YoYo intermittent recovery test was designed to assess the ability of team sport athletes to repeatedly perform intense exercise (Bangsbo, Iaia et al. 2008). The test consists of repeated 2 x 20 m shuttle runs of progressively increasing speeds with 10 s active recovery between efforts until the athlete is no longer able to make the distance in the required time frame (Bangsbo, Iaia et al. 2008).

Maximal oxygen uptake was assessed using a continuous incremental treadmill (Star Trac®, Unisen Inc., USA) test to exhaustion using previously described methods (Slattery, Wallace et al. 2006). Oxygen uptake was measured using the Physio-Dyne® Fitness Instrument Technologies gas analysis system (Quogue, N.Y, USA). The peak running velocity (V_{max}) was taken as the highest treadmill speed maintained for 1 min during the $\dot{V}O_2$max test to prescribe interval training workloads during the HIGH and LOW training periods (Slattery, Wallace et al. 2006).

To determine 3RM for bench press, subjects performed 5-10 repetitions with a light resistance with a 1 min rest period (Gore 2000). The resistance was then progressively increased by 5-15% until the 3RM was established with a 4 min rest period between each trial. No more than five trials were performed for each subject. The results of the 3RM bench press were then used to prescribe the resistance loads for the strength sessions.

During pre-testing session 2, subjects were familiarised with running on the NMT by completing the 15 min protocol used in the match simulation. Subjects were instructed to run at a self-selected pace without performing the sprints maximally. After a 10 min rest period, the peak speed assessment was performed. The peak speed assessment protocol involved a further 4 min of intermittent activity, followed by 3 x 6 s maximal sprints each separated by 2 min of active recovery. The peak sprinting speed was taken as the highest speed obtained during the three sprints and was used to calculate all running speeds used in the simulated team sport match.

Physical training

Subjects completed two, four day blocks of supervised training, in between the match simulations on the NMT. The training load in the HIGH week was designed to be ~50% greater than during the LOW week. Daily training load (AU) was monitored using the session-RPE method which is calculated as the product of training duration (min) and the mean training intensity (rating of perceived exertion CR-10) (Foster, Florhaug et al. 2001). The training consisted of strength sessions, interval running, shuttle runs, agility drills and plyometrics. The training program, session order and estimated internal training load is outlined in Figure 4.2.
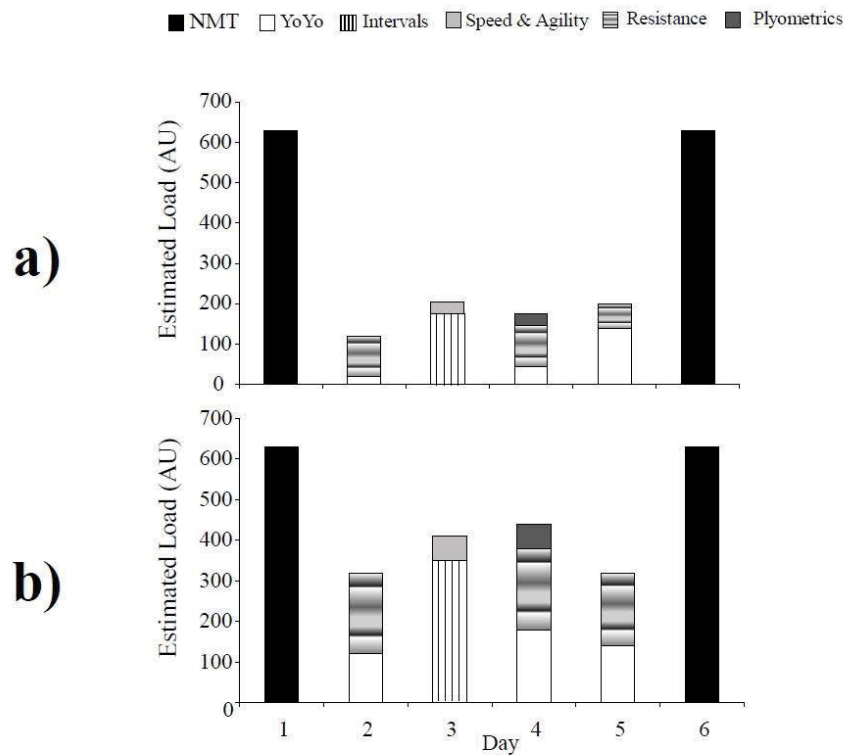
Figure 4.2: Training program for the LOW (a) and HIGH (b) experimental periods.

Subjects completed a general strength program comprised of four lower body exercises (squat, leg extension, leg curl and leg press) and four upper body exercises (bench press, upright row, lat pull down and seated row). Following a warm-up of 8-10 reps with minimal weight, subjects performed either one (LOW) or two (HIGH) sets of 8-10 RM for each exercise. For the interval running sessions, subjects completed either three x (LOW) or six x

(HIGH) 4 min efforts at 85% V_{max} on a motorised treadmill, with 2 min passive recovery between each effort. The intermittent running sessions required subjects to complete YoYo tests to exhaustion (HIGH) or ~50% of the YoYo test distance covered in familiarisation testing (LOW). Agility training consisted of a variety of team sport specific drills (i.e. speed ladders, carioca step, agility poles) which were repeated 3 x (LOW) or 6 x (HIGH). Similarly, for plyometric training, subjects performed either 1 x (LOW) or 2 x (HIGH) sets of 10-20 reps of selected plyometric exercises including, vertical and lateral hops, jumps and hurdles. Twenty four hours of recovery was allowed before each team sport match simulation.

Biochemistry measures

Blood samples were obtained from each subject's antecubital vein pre-exercise, immediately post-exercise and 2 h after the NMT match protocol. Blood samples were collected into three vacutainer tubes, one 10 ml serum separator vacutainer tube and two 7.5 ml tubes lined with EDTA (Becton Dickson, Franklin Lakes, NJ). One EDTA tube was centrifuged immediately after collection at 2000 g for 15 min to separate plasma, which was transferred into 500 µL aliquots and frozen at -80°C until assayed. The second EDTA tube was immediately analysed for hematocrit and haemoglobin using a Sysmex SE 900 (TOA Medical Electronics, Kobe, Japan). Serum creatine kinase (CK), serum lactate dehydrogenase (LDH) and serum C-reactive protein (CRP) were analysed enzymatically using an Architect c8000 System (Abbott Laboratories Inc., Abbott Park, IL, USA).

Enzyme-linked immunosorbent assay kits were used to measure the plasma concentration of monocyte chemoattractant protein-1 (MCP-1) (Human MCP-1 Quantikine Immunoassay, R&D Systems, Minneapolis, USA) (intra-assay CV: 7.4%), interlukin-6 (IL-6) (Human IL-6 Quantikine HS ELISA, R&D Systems, Minneapolis, USA) (intra-assay CV: 7.6%), XO and hypoxanthine (Amplex Red Assay Kit Xanthine/Xanthine Oxidase Assay Kit, Invitrogen, California, USA) (intra-assay CV: 4.2% [XO], 4.5% [hypoxanthine]) according to the manufacturer's instructions. Samples were corrected for exercise-induced plasma volume changes in accordance with the equation developed by Dill and Costill (1974) and methods suggested by Kargotich and others (1998).

Dietary control

To minimise the effects of diet on physical performance, subjects were required to standardise their diet 48 h before and 24 h after each match simulation. Furthermore, two

hours prior to each match simulation, subjects were required to consume a standardised meal, which contained at least 2 g·kg⁻¹·BM of carbohydrate. During each protocol, subjects were allowed to drink water *ad libitum*. Then, after the immediate post-match blood sample, subjects consumed a standard meal that provided an additional 1 g·kg⁻¹·BM of carbohydrate.

Statistical analyses

Before using parametric statistical procedures, the assumption of normality was verified using Mauchy's test of sphericity. An a priori sample analysis revealed 6 pairs of subjects is the minimum required in a matched pair design to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05 (G*Power version 3.1.3, Universität Kiel, Germany). Changes in plasma XO, hypoxanthine, IL-6 and MCP-1 were analysed using a two-way analysis of variance (STATISTICA 6.0, Tulsa, USA) by condition (HIGH and LOW training) and time (pre, post and 2h post-match simulation). If significant main effects were observed without significant interaction, a Scheffe *post hoc* analysis was used to locate the source of the differences. A paired samples t-test was used to determine differences in the pre-exercise measures of muscle damage and inflammation, changes in match performance, and nutrient intake between the experimental periods. Spearman rank order correlations were used to examine the relationships between the measured variables. Data is presented mean ± SD. Statistical significance was accepted at $P < 0.05$.

Results

In the three weeks leading into the study, the mean weekly training load for these subjects was 813 ± 176 AU. During the study period, the training load was greater in the HIGH compared to LOW training period (HIGH 1418 ± 85 AU, LOW 563 ± 42 AU, $P = 0.001$). There were no significant differences in the resting measures for serum CK ($P = 0.75$), serum CRP ($P = 0.69$) or serum LDH ($P = 0.60$) prior to the initial match simulation for each condition (Figure 4.3). However, the increased training load in the HIGH training period elicited a significant increase in the serum concentration of CK ($P = 0.005$) but not serum LDH or serum CRP prior to the second match simulation (Figure 4.3).

Running performance during the simulated team sport match protocol was reduced following the HIGH training period, with a decreased sprint velocity ($P < 0.001$) (Figure 4.4) and total distance covered during the match (HIGH 5495 ± 670 m, LOW 5608 ± 674 m,

P = 0.021). The mean blood lactate during the team sport match simulation was significantly reduced after the HIGH training period compared to the LOW (*P* = 0.03) (Figure 4.5).
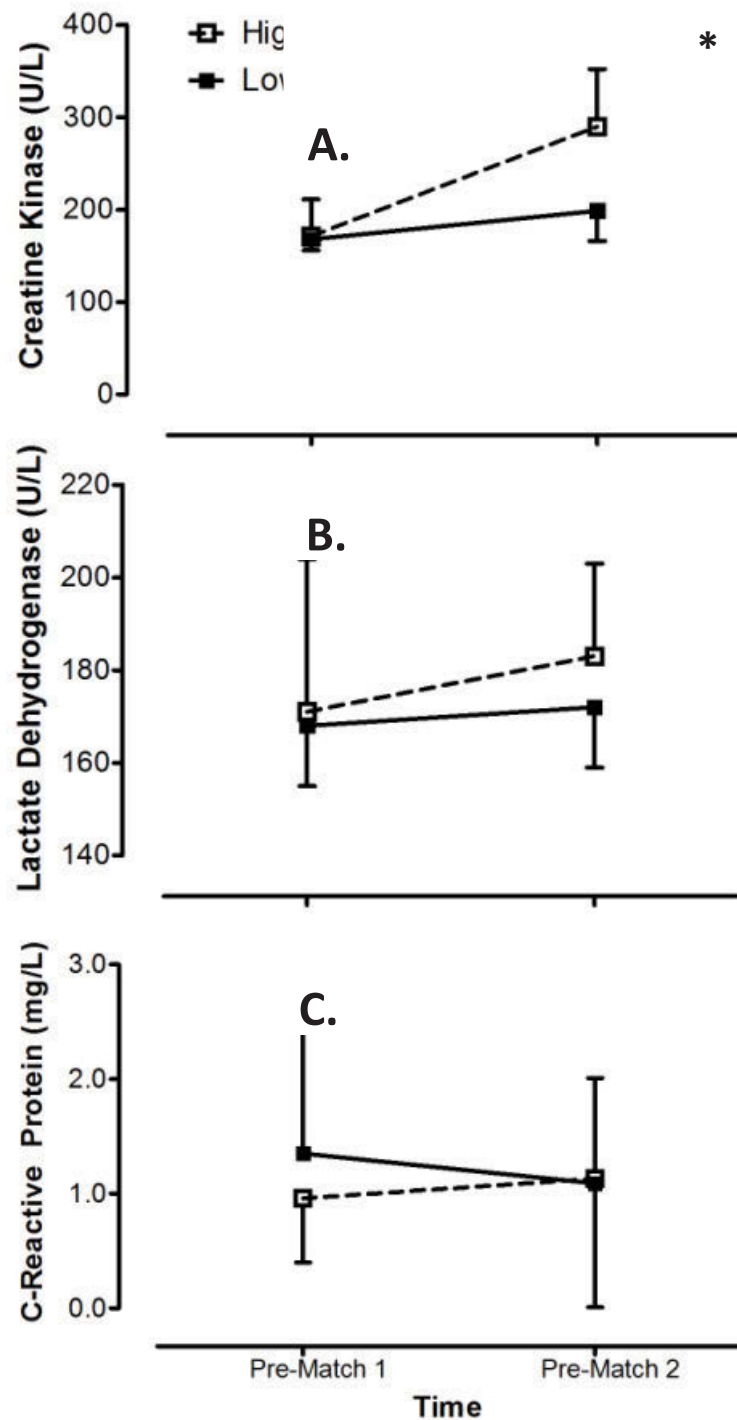
Figure 4.3: Mean (± SD) A. serum creatine kinase, B. serum lactate dehydrogenase and C. serum C-reactive protein concentration prior to each match simulation in the HIGH and LOW conditions. *Significantly different between groups (*P* < 0.05).
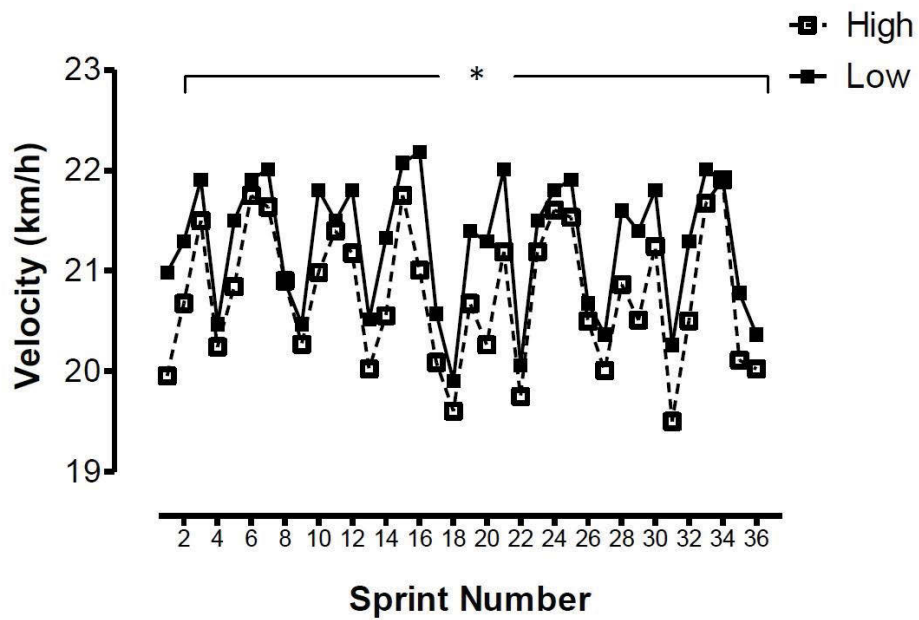
Figure 4.4: Peak sprint velocities reached during sprint throughout the HIGH and LOW match. *Significant difference between groups ($P < 0.01$).
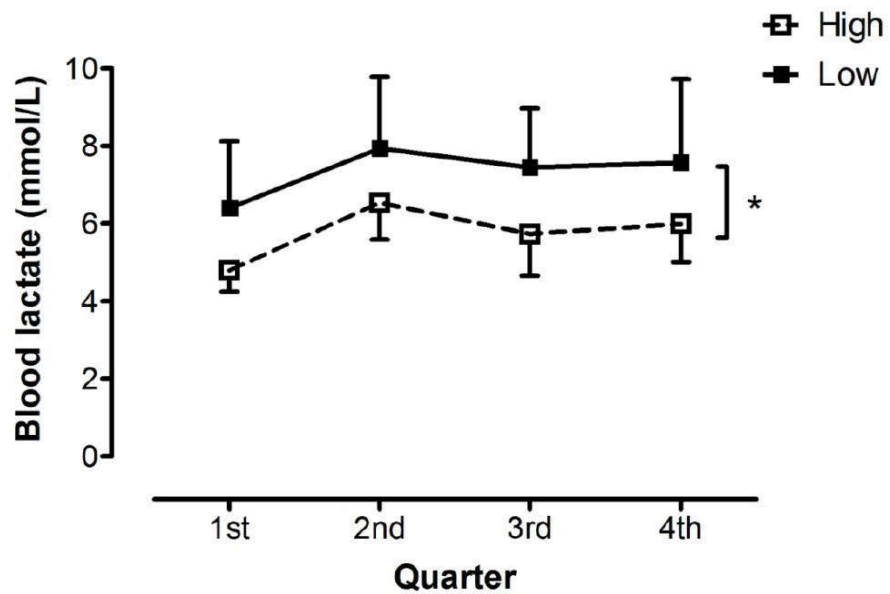
Figure 4.5: Mean (± SD) Capillary blood lactate concentration during the match simulation following the HIGH and LOW training loads *Significantly different between groups ($P < 0.05$).

A significant interaction ($P = 0.004$, $\eta^2 = 0.886$) and time ($P = 0.005$, $\eta^2 = 0.879$) effect was observed plasma XO activity (Figure 4.6a). Post hoc analysis revealed the difference 2 h post-exercise, where plasma XO remained significantly elevated following the HIGH training ($P=0.009$).   Figure 4.6b shows significant main effects in the response of plasma hypoxanthine during the HIGH and LOW match simulations (interaction $P = 0.04$, $\eta^2 = 0.723$; time $P = 0.006$, $\eta^2 = 0.875$). There were significant exercise-induced increases in plasma hypoxanthine, with a greater increase during the LOW match compared to the HIGH ($P = 0.034$). Changes in plasma MCP-1 were observed between the matches for time ($P = 0.002$, $\eta^2 = 0.812$) and training load ($P = 0.009$, $\eta^2 = 0.708$) (Figure 4.7). There was an increase in plasma IL-6 following the match stimulation ($P < 0.001$, $\eta^2 = 0.938$) (Figure 4.6c). However, this change was not significantly different between the HIGH and LOW conditions ($P = 0.059$, $\eta^2 = 0.474$). Post-match plasma hypoxanthine correlated with post-match capillary blood lactate (r = 0.59, $P < 0.05$). No significant difference was found in macro- or micronutrient intake between the two testing periods.
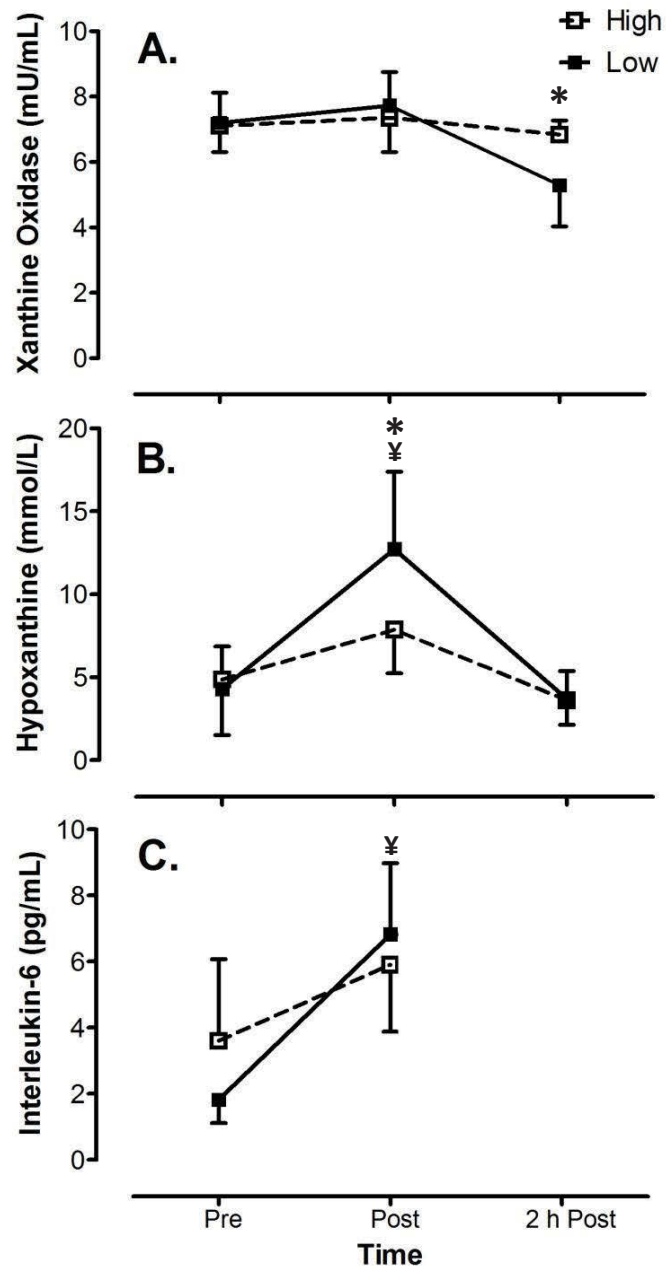
Figure 4.6: Mean (± SD) A. plasma xanthine oxidase, B. plasma hypoxanthine and C. plasma interleukin-6 concentration during the team sport match simulation in the HIGH and LOW conditions. *Significantly different between groups ($P < 0.05$). ¥Significantly different from previous measure ($P < 0.05$).
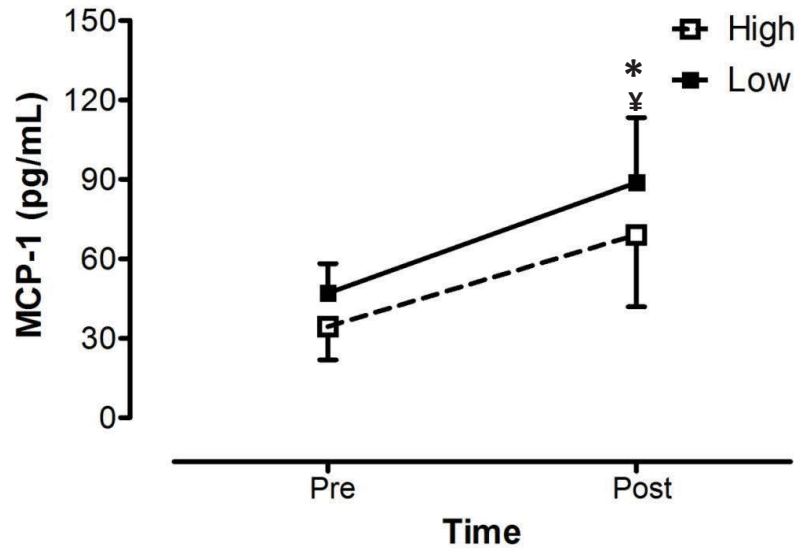
Figure 4.7: Mean (± SD). Pre- to post-match simulation changes in plasma monocyte chemoattractant protein-1 (MCP-1) following the HIGH and LOW training loads. *Significantly different between groups ($P < 0.05$). ¥ Significantly different from previous measure ($P < 0.05$).

Discussion

The present results showed that when team sport athletes continue to complete a heavy physical training load in between matches, anaerobic metabolism and performance capacity may be impaired. This was demonstrated through reduced sprint performance during the match simulation following the HIGH compared to LOW physical training protocol. The HIGH training protocol also induced greater muscle damage, reduced blood lactate and hypoxanthine during the match simulation combined with an increased post-match plasma XO activity.

The training load in the HIGH condition was planned to be ~50% greater than the LOW condition based on increasing training duration whilst maintaining exercise intensity. However, the session-RPE data showed a larger increase in training load measured (60 ± 6%) between the LOW and HIGH conditions. This was due to the higher RPE reported throughout the HIGH physical training (HIGH: RPE 5.7 ± 0.2, LOW: RPE 3.9 ± 0.2). It is likely that the subjects reported elevated RPE scores during the HIGH condition due to a combination of factors such as, insufficient recovery, prior muscle damage, central fatigue, glycogen

depletion and negative psychological attitudes which have all previously been reported to increase the perception of effort during a bout of exercise (Borg, Hassmen et al. 1985).

Following the HIGH training there was a decreased plasma hypoxanthine immediately post-match and a lower capillary blood lactate response throughout the match simulation compared to the LOW match simulation, indicating a reduced activation of the anaerobic metabolism (Hellsten-Westing, Norman et al. 1993). Whilst a lower purine efflux from skeletal muscle during intense exercise is seen as a positive adaptation to sprint training (Stathis, Zhao et al. 1999), it is more likely that the lower plasma hypoxanthine following the HIGH training reflected the reduced exercise intensity and sprint performance during the post-HIGH NMT match simulation (Bianchi, Grossi et al. 1999). This is also supported by the positive correlation between plasma hypoxanthine and capillary blood lactate following the NMT match simulations. These results suggest that the higher plasma hypoxanthine and capillary blood lactate concentrations reached during the LOW match simulation were due to greater utilisation of the anaerobic metabolism. Moreover, it can be speculated that the lower blood lactate concentration during the post-HIGH match occurred as a result of insufficient glycogen stores which may have restricted energy production via glycolytic pathways (Snyder 1998). It is well known that prior muscle damage, as indirectly shown through the significant increase in serum CK concentration pre-HIGH match compared to the LOW match, interferes with the glycolytic response to exercise (Tee, Bosch et al. 2007). Studies have reported increases in insulin resistance and impaired glucose transport for up to 48 h following eccentric exercise, resulting in a reduced glycogen resynthesis rate (Asp and Richter 1996). Therefore, despite consuming a similar amount of carbohydrates during both test periods, the continuation of HIGH compared to LOW training is likely to have reduced muscle glycogen restoration and in turn impaired high-intensity performance capacity during the match simulation (Zehnder, Muelli et al. 2004).

The increased serum CK prior to the HIGH match indicates greater exercise-induced muscle damage from the higher training load, which may have also contributed to the reduced performance. However, the blood inflammatory markers (CRP, XO, IL-6 and MCP-1), were not significantly different between the pre-HIGH and pre-LOW matches. Damage to skeletal muscle fibres and associated inflammation has been shown to cause alterations in the length-tension relationship of the myofibres, resulting in substantial decreases in force production (Proske and Allen 2005). These present findings agree with previous research

that has demonstrated similar reductions in sprint velocity, strength and aerobic running performance with comparable CK concentrations (Twist and Eston 2005).

Exercise-induced increases in XO production have been associated with direct oxidative damage to lipids, DNA and cellular proteins and are involved in the inflammation response (Hellsten, Frandsen et al. 1997; Vina, Gimeno et al. 2000; Gomez-Cabrera, Pallardo et al. 2003). However, when XO activity is suppressed, there is a reduction in markers of muscle damage and inflammation and an inhibition in the up-regulation of antioxidant enzymes (Gomez-Cabrera, Pallardo et al. 2003; Gomez-Cabrera, Borras et al. 2005). Since these factors are important for physiological adaptation, it has been suggested that elevated XO activity following exhaustive exercise is an important part of the training response (Gomez-Cabrera, Borras et al. 2005). In the present investigation, plasma XO activity during the HIGH condition remained significantly elevated 2 h post-exercise compared to the LOW condition where the plasma XO returned to lower than pre-exercise values. This may indicate a continued oxidative response to the match simulation and a greater amount of oxidative damage and inflammation post-HIGH match (Tiidus 1998). The results agree with previous studies that suggest periods of intensified training without sufficient recovery can result in chronic oxidative stress and a reduced performance capacity (Schippinger, Wonisch et al. 2002; Finaud, Scislowski et al. 2006). Collectively, these findings highlight the importance of appropriate between-match recovery practices for team sport athletes.

Changes in IL-6 release during exercise may be important as it has a dual role in regulating certain endocrine and immune responses to physical training (Pedersen, Steensberg et al. 2003). Elevations in basal IL-6 of only 5 pg ml^{-1}·have been reported as a marker of training fatigue and associated with both immune system suppression and increased CK levels in endurance-trained triathletes undergoing intensive sprint training (Robson-Ansley, Blannin et al. 2007). In this study however, whilst three of the seven subjects following the HIGH training load had resting plasma IL-6 concentrations above 5 pg ml^{-1}, there were no differences in plasma IL-6 between the two conditions. Similarly, there were also no differences in resting plasma MCP-1 concentration between the training conditions. It is likely that a period of greater than the 4 days of intense physical training used in the present study would be required to cause significant alterations in an athlete's basal inflammatory response.

Collectively, the present findings demonstrate the strenuous nature of high-intensity, intermittent activity and highlight the importance of adequate recovery between matches. Four days of HIGH training, where each training session was ~60 min, was sufficient to increase markers of muscle damage, reduce glycolytic response and impair sprint capacity during the team sport match simulation. Therefore, interventions to either reduce initial elevations in plasma XO, such as administration of allopurinol (Gomez-Cabrera, Pallardo et al. 2003) or promote recovery from intense exercise, such as contrast water therapy (Rowsell, Coutts et al. 2011) may be warranted.

CHAPTER FIVE

Evaluating the effect of acute changes in training load on select endocrinological and oxidative damage markers in team sport players

Abstract

Acute changes in the resting and post-exercise levels of select hormonal and oxidative damage markers may provide information on the fatigue state of team sport athletes following short-term periods of intensive training. Using a randomised controlled, crossover experimental design, seven team sport athletes completed a 60 min high-intensity intermittent running protocol on a non-motorised treadmill after either 4 days of increased (HIGH) or reduced (LOW) training loads. Venous blood samples were taken pre-, post- and 2 h post-high-intensity intermittent running protocol to assess plasma measures of growth hormone, thyroid stimulating hormone, free triiodothyronine and free thyroxine. Urine samples were collected at the same time points to measure 15-isoprostane F_{2t} concentration (F_2-isoprostane). Participants completed a significantly higher training load in the HIGH compared to LOW condition (HIGH 1418 ± 85 AU, LOW 563 ± 42 AU, $P < 0.01$, d = 5.86). Following the HIGH training load, performance capacity was reduced during the high-intensity intermittent running protocol compared to the LOW match simulation. Significant exercise-induced changes in plasma GH ($P < 0.001$), plasma TSH ($P < 0.05$) and urinary F_2-isoprostane ($P < 0.01$) were observed. However, there was no difference in these measures between the HIGH and LOW training conditions. These results show that the greater physical training load reduced running performance, but did not increase the level of oxidative damage or alter the circulating levels of growth or thyroid hormones. This suggests that the oxidative and endocrinological systems can adequately maintain homeostatic regulation during acute periods of intensified physical training.

Key Words: Fatigue, F_2-isoprostane, growth hormone, thyroid hormones.

Introduction

To maintain strength, speed and aerobic fitness qualities developed during the pre-season, team sport athletes are often required to continue intensive physical training throughout the competition period (Caldwell and Peters 2009). It can be difficult to determine the appropriate training dose and recovery periods needed to perform optimally in matches on a weekly basis. An insufficient training stimulus may have a detraining effect, leading to reductions in fitness components and lead to decreased physical performance. However, if strenuous exercise is performed when individuals have not fully recovered from the prior match, an increased level of fatigue may occur. Previous research has examined the time-course of recovery in physical performance, oxidative stress and hormonal markers following team sport matches (Ascensao, Rebelo et al. 2008; Andersson, Bohn et al. 2010; McLellan, Lovell et al. 2010). The progressive changes in these measures throughout an entire competitive season have also been investigated (Finaud, Scislowski et al. 2006; Martinez, Seco Calvo et al. 2010). In general, these studies have reported reductions in various physical performance measures (i.e. sprint ability and jump capacity) and an associated increase in markers of oxidative damage and hormonal dysregulation, which typically remain impaired for 20–72 h following matches. Indeed, the repeated exercise stress of weekly matches has been shown to accumulate throughout the season and may lead to a state of non-functional overreaching (Moore, Timmerman et al. 2005). However, the acute effects of manipulations in training load in between team sport matches on changes in oxidative damage, hormonal measures and subsequent physical performance, is less well known. This is an important issue to address as many sporting teams often play two matches in a week or have periods of competition with relatively short recovery periods (i.e. < 5 days) between matches. Such errors in training periodisation during this time may impact on player recovery and subsequent exercise performance.

Athletes completing high training loads with limited recovery periods may be in a state of chronic oxidative stress (Finaud, Scislowski et al. 2006; Margonis, Fatouros et al. 2007), which could contribute to increased levels of fatigue from physical training. Indeed, Margonis et al. (2007) demonstrated a dose-response relationship between training load and basal urinary 15-isoprostane F_{2t} concentration (F_2-isoprostane) measures. Compared to baseline, urinary F_2-isoprostane concentration increased 2.4, 4.0 and 7.0 fold during light, intense and overtraining periods, respectively. Similarly, Finaud et al. (2006) observed a significantly greater amount of oxidative damage measured via conjugated dienes (67%) in 17 male

professional rugby players when training volume was increased by 28%. These increases in exercise-induced oxidative damage can cause extensive cellular degradation that may manifest as decreased skeletal muscle function, reduced immunity, allergies and/or hormonal dysregulation (Clarkson and Thompson 2000). Based on these findings, it may be beneficial to monitor changes in oxidative damage to assess whether an individual is tolerating the prescribed physical training load. Furthermore, monitoring fluctuations in the hormonal response to acute changes in training load may also assist in the assessment of training-induced fatigue.

The up-regulation of the hypothalamic-pituitary-adrenal / thyroid axis plays an integral role in maintaining a homeostatic balance during exercise (Steinacker, Brkic et al. 2005). However, investigations assessing the anabolic / catabolic balance via changes in testosterone and cortisol in a field setting with high level team sport athletes have shown conflicting results (McLean, Coutts et al. 2010; McLellan, Lovell et al. 2010; Crewther, Cook et al. 2011). It may therefore be of interest to examine changes in the concentration of other hormones involved in the regulation of metabolic and anabolic responses to physical training such as the thyroid hormones and growth hormone (GH) (Moore, Timmerman et al. 2005; Stokes, Nevill et al. 2005). Specifically, the release of thyroid hormones during physical activity can increase the functional capacity of skeletal muscle via the increased expression of myosin heavy chain proteins (Wahrmann, Fulla et al. 2002) and enhanced mitochondrial biogenesis (Casas, Pessemesse et al. 2008). It has also previously been demonstrated that the thyroid hormones have a permissive effect on the physiologic actions of GH [i.e. increases in protein synthesis, enhanced lipolysis, greater glucose uptake and increased collagen synthesis] (Giustina and Wehrenberg 1995). A dysregulation in either hormonal pathway may attenuate the anabolic and metabolic adaptive processes to exercise. Changes in plasma thyroid hormones or GH concentration could provide an early indication of training-induced fatigue and exercise maladaptation in team sport athletes.

At present, relatively little is known about the impact of altering training dose during the recovery period from intense exercise or competition on subsequent physical performance. This research was designed to examine the acute changes in resting and post-exercise level of select hormonal and oxidative damage markers following 4 days of either an elevated or a reduced training load. Our hypothesis was that the acute increase in training stimuli would be insufficient to alter resting plasma hormonal concentrations and level of oxidative stress.

However, it was anticipated that a higher training load would induce a greater level of fatigue, which may exacerbate oxidate damage, affect hormonal regulation and reduce performance during a high-intensity intermittent running protocol.

Methods

Participants

Seven trained male team sport athletes (age 20.3 ± 2.4 y, body mass 80.5 ± 8.1 kg) volunteered for the investigation. Participants played at a club to regional level in their sport and completed between 4–8 physical training sessions per week. Prior to the commencement of the study participants were informed of the potential risks involved and gave written consent. The research was approved by the University of Technology, Sydney (UTS) Human Research Ethics Committee and conducted in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans.

Experimental Design

A randomly assigned, crossover experimental design was used to determine the effects of prior training load on select measures of oxidative stress and hormonal regulation. Prior to the testing period, pre-testing and familiarisation were completed. Participants performed a 60 min high-intensity intermittent running protocol on a non-motorised treadmill (NMT; Woodway Force 3.0, Waukesha, WI) prior to and following 4 d of either increased (HIGH) or reduced (LOW) training loads. The two 6 d testing periods were separated by a one week washout period. Participants were required to complete a training diary for the week prior to the first experimental period to ensure a similar training load preceding each testing week. The participants then repeated the same training during the washout period between the two testing weeks.

High-intensity intermittent running protocol

The 60 min high-intensity intermittent running protocol was based on time-motion analyses to closely replicate the physiologic demands, which occur during team sport match-play (Sirotic and Coutts 2007). Six running speeds, based on a percentage of each participant's peak sprinting speed were used in the protocol; stand still (0%), walk (20%), jog (35%), run (45%), fast run (65%) and sprint (100% or maximal effort). Customised software was used to generate audio and visual signals for change of speed and real-time velocity

feedback to ensure individual target speeds were met. The same 15 min protocol was repeated four successive times with a 10 min rest period between the second and third repetition to mimic a half-time break. The NMT used in this investigation has previously been described in detail (Sirotic and Coutts 2007). The treadmill running belt velocity, distance, and horizontal forces were collected at a sampling rate of 10 Hz using the XPV7 PCB interface (Fitness Technology, Adelaide, Australia) and analysed using the Force 3.0 Software (Innervations, Joondalup, Australia). The reliability of the treadmill system in our laboratory has previously been established (technical error of measurement (TEM) = 1.9%; intraclass correlation (ICC) = 0.93) (Sirotic and Coutts 2007).

Familiarisation and physical training

The participants were familiarised with the NMT protocol during their first visit to the laboratory. A second familiarisation and peak speed assessment on the NMT and the YoYo Intermittent recovery test (level 1) were completed during pre-testing session 2. and completed a In a second familiarisation session peak speed was assessed (3 x 6 s maximal sprints each separated by 2 min of active recovery) on the NMT and the YoYo Intermittent recovery test (level 1) was performed (Bangsbo, Iaia et al. 2008). Throughout the study, each physical training session completed between the high-intensity intermittent running protocols was supervised by the investigator. Daily training load (AU) was monitored using the session-RPE method (Foster, Florhaug et al. 2001) which is calculated as the product of training duration (min) and the global training intensity (rating of perceived exertion CR-10) (Borg, Hassmen et al. 1985). The training load in the HIGH week was designed to be ~50% greater than during the LOW week (Figure 5.1). Training consisted of strength sessions, interval running, shuttle runs, agility drills and plyometrics.
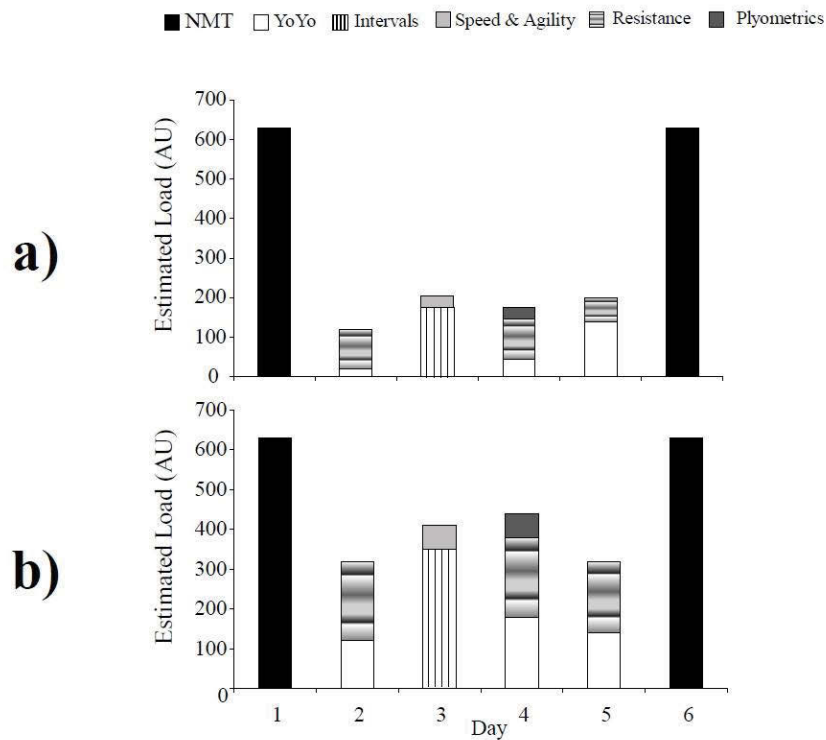
Figure 5.1: Training program for the LOW (a) and HIGH (b) experimental periods.

Interval running sessions, were completed at ~92% HR_{max} on a motorised treadmill (Star Trac, Unisen Inc. Irvine, CA). Participants completed either 3 x (LOW) or 6 x (HIGH) 4 min efforts, with a 2 min passive recovery between each effort. The intermittent running sessions required participants to complete YoYo tests to exhaustion (HIGH) or ~50% of the YoYo test distance covered in familiarisation testing (LOW). Agility training consisted of a variety of team sport specific drills, which were repeated 3 times (LOW) or 6 times (HIGH). Plyometric training was performed either 1 set (LOW) or 2 sets (HIGH) of 10-20 repetitions of selected plyometric exercises including, vertical and lateral hops, jumps and hurdles. Strength training consisted of 1 (LOW) or 2 (HIGH) sets of 8-10 RM for lower body (squat, leg extension, leg curl, leg press) and upper body exercises (bench press, upright row, lat pull down, seated row).

Biochemistry measures

Blood and urine samples were obtained pre-exercise, immediately post-exercise and 2 h after the NMT high-intensity intermittent running protocol completed after both the HIGH and LOW training conditions. Samples were centrifuged immediately at 2000 g for 15 min to separate plasma. Plasma was then transferred into 500 μL aliquots and frozen at -80°C until assayed for growth hormone (GH) using an enzyme-linked immunosorbent assay (ELISA) kit (Human Growth Hormone Quantikine Immunoassay, R&D Systems, Minneapolis, USA) (intra-assay CV: 5.4%). Serum concentration of thyroid stimulating hormone (TSH), free triiodothyronine (FT_3) and free thyroxine (FT_4) were analysed enzymatically using an Architect c8000 System (Abbott Laboratories Inc., Abbott Park, IL, USA). Exercise-induced plasma volume changes were calculated in accordance with the equation developed by Dill and Costill (1974) and methods suggested by Kargotich and others (1998). Urinary samples were stored at -80°C until assayed using an ELISA kit to measure 15-isoprostane F_{2t} concentration (F_2Isoprostane) (Oxford Biomedical Research, MI, USA) (intra-assay CV: 5.8%) which was then normalised against urinary creatinine concentration (Oxford Biomedical Research, MI, USA) according to the manufacturer's instructions.

Dietary control

Participants were required to record nutritional diaries and standardise their diet 48 h before, and 24 h after each high-intensity running protocol. In addition, 2 h prior to each run, participants were required to consume a standardised meal, which contained at least 2 $g \cdot kg^{-1} \cdot BM$ of carbohydrate. Participants drank water *ad libitum* during the simulation. Then, after the immediate post-exercise blood sample, participants consumed another standard meal that provided an additional 1 $g \cdot kg^{-1} \cdot BM$ of carbohydrate.

Statistical analyses

Before using parametric statistical procedures, the assumption of sphericity was verified with Mauchy's test of sphericity. An a priori sample analysis revealed 6 pairs of subjects is the minimum required in a matched pair design to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05 (G*Power version 3.1.3, Universität Kiel, Germany). Changes in F_2Isoprostane, GH, TSH, FT_3 and FT_4 were analysed using a two-way analysis of variance (STATISTICA 6.0, Tulsa, USA) by condition (HIGH and LOW training) and time (pre, post and 2 h post-high-intensity intermittent running protocol). If significant

main effects were observed, a Scheffe *post hoc* analysis was used to locate the source of the differences. Effect size was calculated using Cohen's *d*, with values of 0.2, 0.5 and > 0.8 were considered small, moderate and large, respectively (Cohen 1988). A non-parametric Wilcoxon Sign matched pairs test was used to determine differences in training load, nutrient intake and match performance variables. Statistical significance was accepted at *P* < 0.05.

Results

A greater training load was completed during the HIGH training period (HIGH 1418 ± 85 AU, LOW 563 ± 42 AU, *P* < 0.01, *d* = 5.86). There was a decreased peak sprint velocity (HIGH 20.76 ± 0.28 km•h^{-1}, LOW 21.26 ± 0.22 km•h^{-1}; *P* < 0.01, *d* = -0.69), reduced sprint distance (HIGH 536 ± 27 m, LOW 562 ± 30 m; *P* < 0.01, *d* = -0.94) and total distance covered during the high-intensity intermittent running protocol following the HIGH training (HIGH 5495 ± 670 m, LOW 5608 ± 674 m; *P* < 0.05, *d* = -0.17). No significant difference was found in macro- or micronutrient intake between the two testing periods.

No main effect was found in normalised F_2-isoprostane between conditions (*P* = 0.76, *d* = 0.25) but the difference from pre- to post-exercise was significant (*P* = 0.01, *d* = -0.99) as shown in Figure 5.2. Similarly, no main effect was observed in GH between the training conditions (*P* = 0.47, *d* = 0.36) (HIGH pre-exercise 0.4 ± 0.2 pg•ml^{-1}, post-exercise 9.9 ± 5.8 pg·ml^{-1}; LOW pre-exercise 0.3 ± 0.1 pg·ml^{-1}, post-exercise 7.8 ± 4.8 pg·ml^{-1}). There were also no main effects in TSH (*P* = 0.89), FT_3 (*P* = 0.94) or FT_4 (*P* = 0.69) as shown in Figure 5.3. Significant exercise-induced increases in plasma GH concentration (*P* < 0.001, *d* = -1.54) and serum TSH (*P* < 0.05, *d* = -0.95) were observed.
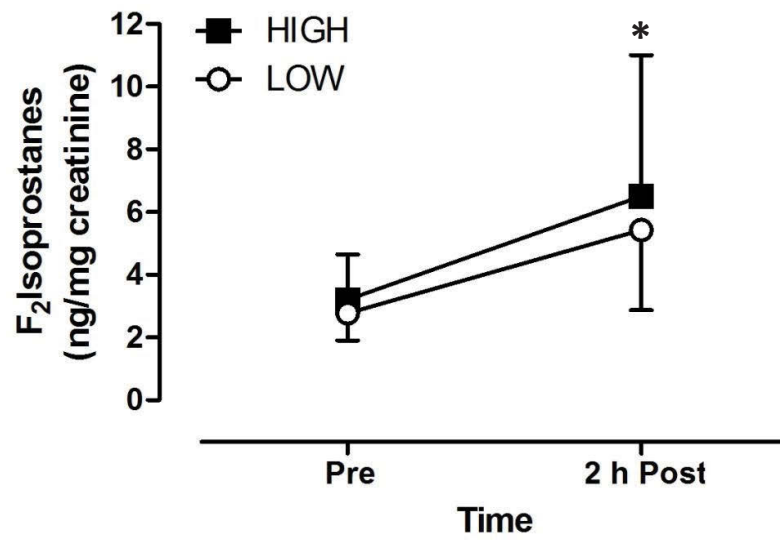
Figure 5.2: Mean ($\pm$ SD) F_2-isoprostane during the high-intensity intermittent running protocol following the HIGH and LOW training conditions. *Significantly different time effect, pre-post exercise ($P < 0.05$).

Figure 5.3: Mean (± SD) A. serum thyroid stimulating hormone (TSH), B. serum free triiodothyronine (FT_3) and C. serum free thyroxine (FT_4) during the high-intensity intermittent running protocol in the HIGH and LOW conditions. *Significantly different time effect, pre-post exercise ($P < 0.05$).

Discussion

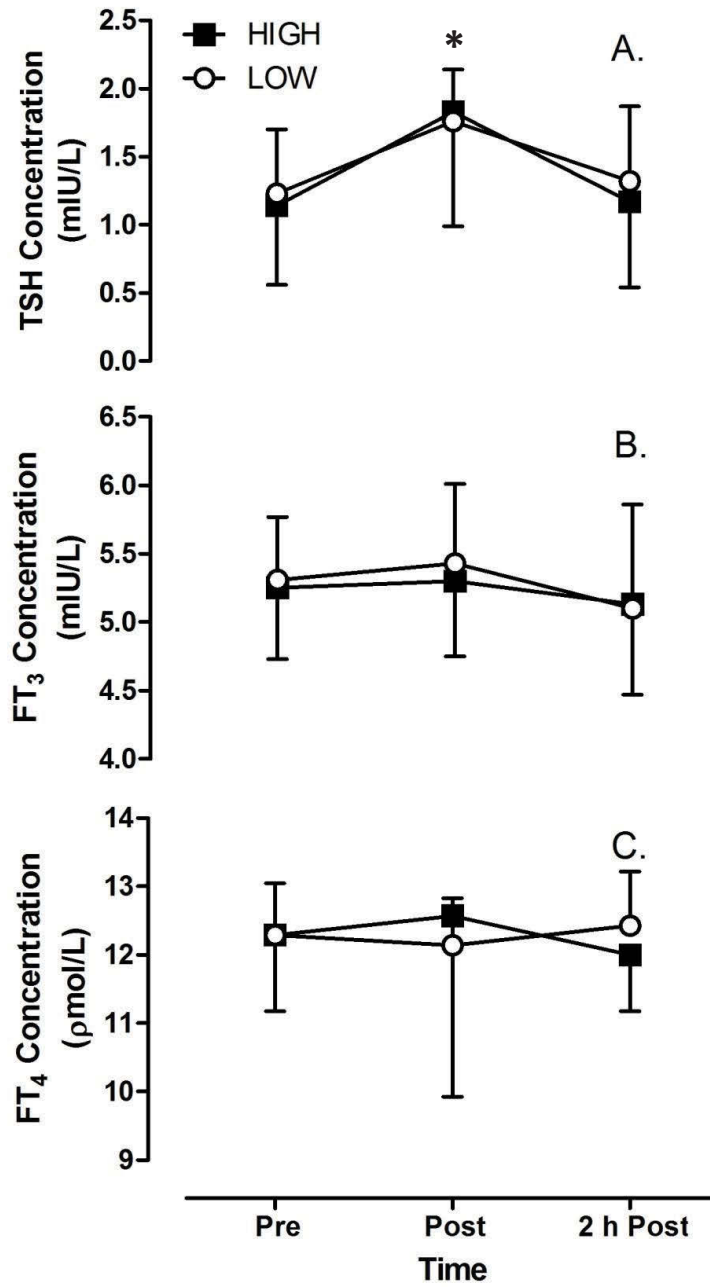Alterations in neuroendocrine activity and increased levels of oxidative damage have been used in team sport athletes to assess long term changes in an individual's state of fatigue and recovery (Finaud, Scislowski et al. 2006; Coutts, Reaburn et al. 2007). The present investigation was designed to examine whether these measures are also sensitive to acute manipulations in physical training load and are reflective of performance capacity. Running performance during a NMT high-intensity intermittent running protocol was reduced following a HIGH compared to LOW training load. However, no significant effect of the 152% greater training load was observed in either resting or post-exercise urinary F_2-isoprostane concentration, GH or thyroid hormone parameters. These results suggest that changes in these measures do not reflect reductions in performance capacity due to a short-term (4 d) increase in intensive physical training.

Monitoring changes in urinary F_2-isoprostane is a promising non-invasive method that can indicate when an athlete is in a state of oxidative stress. F_2-isoprostanes are derived from the free radical catalysed peroxidation of arachidonic acid and are the preferred method to assess oxidative damage *in vivo* (Nikolaidis, Kyparos et al. 2011). Fluctuations in basal urinary measures have been shown to mirror training-induced performance reductions (i.e. jump tests) and correlate closely with changes in plasma markers of oxidative damage (Margonis, Fatouros et al. 2007). In the present investigation, resting urinary measures of F_2-isoprostane were not elevated in response to the HIGH training condition. These findings suggest that basal F_2-isoprostane may be more useful to detect the accumulative effect of increases in training load, rather than transient perturbations. In comparison to the 4 d increased training load examined in the current study, previous research utilised a 3 week intensified training period to elicit an increase in basal F_2-isoprostane concentration (Margonis, Fatouros et al. 2007). Likewise, the high-intensity intermittent running protocol induced a similar amount of post-exercise oxidative damage following both the HIGH and LOW training periods. Prior investigations have demonstrated exercise-induced increases in plasma and urinary F_2-isoprostane following prolonged endurance exercise (Steensberg, Morrow et al. 2002; Nieman, Henson et al. 2004; Nikolaidis, Kyparos et al. 2011). However, little is known about the effect of a short-term block of intensified training on F_2-isoprostane concentration. Studies have investigated the time-course change of urinary allantoin, a by-product of the non-enzymatic oxidation of urate, (Shing, Peake et al. 2007) and urinary 8-hydroxydeoxyguanosine (8-OHdG), an indicator of oxidative DNA damage (Radak, Pucsuk et

al. 2000), to assess oxidative stress during 3-4 consecutive days of high-intensity exercise. Interestingly, both papers reported increases in urinary oxidative damage measures following the initial exercise bout, which progressively declined throughout the investigation. This blunted oxidative response with repeated strenuous bouts of exercise was viewed as an adaptive response within the oxidant-antioxidant system (Radak, Pucsuk et al. 2000). It is possible that the HIGH training load induced a similar adaptive response in the current investigation. Further research is warranted on the usefulness of urinary F_2-isoprostane as a non-invasive measure of exercise-induced oxidative damage in team sport athletes.

There is still no clear consensus as to the dose-response relationship between physical training loads and hormonal regulation. This may be due to the highly variable hormonal profile between individuals which can be influenced by a myriad of exercise related factors (i.e. exercise duration, intensity, mode) and non-exercise related factors (i.e. psychological stress, temperature, nutrition, hypoxia, hydration status, circadian rhythm) (Viru, Hackney et al. 2001). In the present study, changes in resting plasma hormonal concentrations were not expected. This hypothesis was based on the findings of previous investigators whereby several weeks / months of intensified training have not altered the resting plasma measures of hormones associated with the hypothalamic-pituitary axis (Urhausen, Gabriel et al. 1998). For example, Rietjens et al. (2005) increased the training volume of seven well-trained cyclists from 420 to 870 min·wk^{-1} for two weeks and did not observe any significant difference in pre-exercise plasma GH or TSH. In support, the findings of the present investigation showed that the HIGH training load did not induce any significant difference in pre-exercise plasma GH, FT_3, FT_4 or TSH concentration. It is therefore proposed that monitoring changes in resting measures of these hormones is not useful to assess the relative fatigue imposed by physical training between team matches.

It has been reported that athletes in a fatigued *vs.* non-fatigued state have a dissimilar hormonal response to strenuous exercise (Meeusen, Nederhof et al. 2010). For instance, Ronsen et al. (2001) showed an augmented GH response to a repeated endurance exercise session (65 min cycling at 75% $\dot{V}O_2$max with 3 h recovery in between bouts) in nine elite male endurance cyclists. However, a greater GH release with pre-fatigue has not been consistently shown (Viru, Hackney et al. 2001). No difference in plasma concentration of GH was observed when 12 endurance trained male runners performed a 10 min cycle at 70% $\dot{V}O_2$max on a cycle ergometer, with or without a prior 2 h run (Viru, Hackney et al. 2001).

Similarly, no change in post-exercise GH concentration occurred when 11 weight-trained men underwent an overtraining period (Fry, Kraemer et al. 1998). This is in agreement with the current results where, despite a reduction in running performance after HIGH physical training, no significant difference was observed in post-exercise plasma GH conditions compared to LOW physical training. This may be an indication that there is a volume / intensity threshold whereby additional training loads may decrease running performance, but are not severe enough to cause hormonal dysregulation.

Disturbances in thyroid hormone regulation (i.e. hypo- and hyperthyroidism) can result in a reduced capacity to perform physical activity (Steinacker, Brkic et al. 2005). An overproduction of thyroid hormones (hyperthyroidism) has been shown to increase oxidative stress (Asayama and Kato 1990), whilst an under-production of thyroid hormones (hypothyroidism) has been shown to cause excessive fatigue and decreases in muscle strength (Steinacker, Brkic et al. 2005). It is therefore important for athletes undergoing intensified training periods to remain in a euthyroid state. Most research on thyroid function has focused on changes in the resting measures of the hormone during prolonged training periods (Simsch, Lormes et al. 2002). The present study examined the effect of varied training loads on the post-exercise changes in circulating TSH, FT_3 and FT_4. Prior to the commencement of the training intervention, it was anticipated that participants undertaking the HIGH condition may exhibit a blunted thyroid hormone response. This hypothesis was based on previously demonstrated reductions in TSH in individuals undergoing heavy physical training (Simsch, Lormes et al. 2002). However, the results of the present study showed that a 4 d intensified physical training period had no effect on exercise-induced changes in thyroid hormone regulation. In contrast to the initial hypothesis, exercise-induced changes in thyroid hormones are not recommended to assess acute differences in training induced fatigue / recovery balance. Monitoring changes in these hormones to assess recovery from matches in team sports would also have limited practical utility.

The present investigation shows that measures such as urinary F_2-isoprostane and plasma GH, TSH, FT_3 and FT_4 may be more suited to assessing chronic rather than acute effects of physical training load in team sport athletes. However, it is acknowledged that the lack of statistical differences in the biochemical variables may be due to the high inter-individual variability of hormonal responses, which may limit the applicability of these results to a wider population. Nonetheless, it is likely that disturbances in the hormones within the

hypothalamic-pituitary axis are more reflective of an individual's overall health and well-being, as opposed to being sensitive markers for acute exercise-induced fatigue.

CHAPTER SIX

The effect of N-acetylcysteine on cycling performance following intensified training in well-trained triathletes: a double blind randomised placebo controlled study

Abstract

This investigation examined the ergogenic effect of short-term oral N-acetylcysteine (NAC) supplementation and the associated changes in redox balance and inflammation during intense training. A double blind randomised placebo-controlled crossover design was used to assess 9 days of oral NAC supplementation (1200 mg/day) in 10 well-trained triathletes. For each supplement trial (NAC and placebo), baseline venous blood and urine samples were taken and a pre-supplementation cycle ergometer race simulation was performed. Following the loading period, further samples were collected pre-exercise, post-exercise, 2 h and 24 h following the post-supplementation cycle ergometer race simulation. Changes in total antioxidant capacity (TAC), ferric reducing ability of plasma (FRAP), reduced glutathione (GSH), oxidised glutathione (GSSG), thiobarbituric acid-reactive substances (TBARS), interlukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), nuclear factor- κB (NF-κB) and urinary 15-isoprostane F_{2t} concentration (F_2-isoprostane) were assessed. The experimental procedure was repeated with the remaining supplement after a 3 week washout. Eight participants completed both supplementation trials. NAC improved sprint performance during the cycle ergometer race simulation ($P < 0.001$, $\eta^2_p = 0.03$). Supplementation with NAC also augmented post-exercise plasma TAC ($P = 0.005$, $\eta^2_p = 0.19$), reduced exercise-induced oxidative damage, [plasma TBARS ($P = 0.002$, $\eta^2_p = 0.22$); urinary F_2-isoprostane ($P = 0.010$, $\eta^2_p = 0.431$)]; attenuated inflammation [plasma IL-6 ($P = 0.002$, $\eta^2_p = 0.22$); MCP-1 ($P = 0.012$, $\eta^2_p = 0.17$)] and increased post-exercise NF-κB activity ($P < 0.001$, $\eta^2_p = 0.21$). Oral NAC supplementation improved cycling performance via an improved redox balance and promoted adaptive processes in well-trained athletes undergoing strenuous physical training.

Key words: Antioxidant, oxidative stress, inflammation, nuclear factor- κB.

Introduction

The oxidant-antioxidant balance during exercise has been shown to influence skeletal muscle contractile capacity (Reid, Stokic et al. 1994) and adaptation to exercise (Gomez-Cabrera, Borras et al. 2005). Moderate exercise-induced elevations in reactive oxidative species (ROS) within skeletal muscle can enhance contractile function and play an important role in the reduction-oxidation (redox) activation of numerous cellular signalling cascades which promote skeletal muscle gene expression and integrity (Powers, Duarte et al. 2010). However, during unaccustomed, high intensity, prolonged or strenuous exercise, the antioxidant defence system may be unable to buffer the oxidant concentration within skeletal muscle. This may lead to an accumulation of ROS which has been shown to inhibit excitation-coupling and contribute to the development of fatigue in skeletal muscle (Reid, Stokic et al. 1994). It has been proposed that the consumption of exogenous antioxidant compounds may increase the total antioxidant capacity of the intra and extra cellular milieu. This improved antioxidant status may then assist in the maintenance of an optimal redox balance during exercise and in turn promote skeletal muscle performance.

N-acetylcysteine (NAC) is a thiol containing compound which acts to minimise the exercise-induced oxidative insult through its actions as a cysteine donor in the maintenance of glutathione homeostasis and via direct scavenging of ROS (Cotgreave 1997). Several studies have demonstrated acute improvements in both aerobic and resistance-based performance tasks when participants were supplemented either orally or by infusion with the antioxidant NAC (Reid, Stokic et al. 1994; Medved, Brown et al. 2004; Matuszczak, Farid et al. 2005; Cobley, McGlory et al. 2011). Experimental evidence suggests that NAC supplementation exerts an acute ergogenic effect through the minimisation of oxidant interference in the activity of key ion transporters and ion channel proteins (Medved, Brown et al. 2004) and through the improved regulation of calcium release within contracting myofibres (Andrade, Reid et al. 2001). Recent *in vivo* research on the force-frequency characteristics of mouse diaphragm fibre bundles has also demonstrated that NAC treatment promotes fatigue resistance by delaying the slowing of cross-bridge detachment (Ferreira, Campbell et al. 2011). Additionally, NAC may improve hemodynamics via a secondary vasodilatory effect during exercise to promote blood flow and muscle perfusion (Reid, Stokic et al. 1994). NAC has also been shown to have a role in the modulation of haematological parameters and erythropoietin (EPO) secretion, which would contribute to an improved performance capacity (Zembron-Lacny, Slowinska-Lisowska et al. 2010). For instance, an

eight day supplementation period with NAC lead to significant increases in plasma EPO (+26%) and haemoglobin (+9%) in eight healthy males compared to a control group (Zembron-Lacny, Slowinska-Lisowska et al. 2010). This data suggests that supplementation with NAC can induce several physiological changes which would enhance competitive performance. However, a beneficial effect of NAC during exercise has not been consistently demonstrated (Medved, Brown et al. 2003). Further research is required to firmly establish the ability of NAC to improve athletic performance in a practical sports setting.

A reduction in oxidant release during skeletal muscle contractions observed with NAC supplementation may also impact the inflammatory response to exercise and alter the expression of redox sensitive gene transcription factors (Petersen, McKenna et al. 2011). Previously, NAC supplementation has been shown to promote the up-regulation of anti-inflammatory cytokines and minimise skeletal muscle injury following fatiguing contractile activity (Pinheiro, Vitzel et al. 2012). This reduction in oxidative damage to contractile proteins within the myofibres may therefore prevent the decline in force production associated with exercise-induced muscle damage and increase the capacity for athletes to continue to perform strenuous physical training (Proske and Allen 2005). However, a decrease in exercise-induced skeletal injury and / or inflammation has not been consistently shown with NAC supplementation (Childs, Jacobs et al. 2001; Cobley, McGlory et al. 2011). Several investigations have reported elevations in inflammatory measures and muscle damage with antioxidant supplementation (Childs, Jacobs et al. 2001; Teixeira, Valente et al. 2009). This has led to the hypothesis that antioxidant supplementation may suppress the activation of the inflammatory cascade following exercise-induced muscle damage and cause interference with the subsequent repair and remodelling of skeletal tissue (Teixeira, Valente et al. 2009).

The redox sensitive transcription factor, nuclear factor–kappa B (NF-κB), plays a key role in the regulation of genes involved in both the inflammatory cascade [i.e. interlukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1)] and within the antioxidant enzyme system (Kramer and Goodyear 2007). Studies have reported elevations in NF-κB activity following both isolated muscle contractions and whole body exercise (Cuevas, Almar et al. 2005; Gomez-Cabrera, Borras et al. 2005). Due to the large number of genes that are up-regulated via the NF-κB pathway, it has been proposed that NF-κB may be a central mediator of the physiological adaption to exercise stimuli (Gomez-Cabrera, Borras et al. 2005; Powers,

Duarte et al. 2010). Indeed, inhibition of NF-κB phosphorylation with supplementation [i.e. allopurinol (Gomez-Cabrera, Martinez et al. 2006) and NAC (Farid, Reid et al. 2005)] has been shown to blunt positive exercise-induced physiological changes from occurring. However, not all investigations have reported an inhibition of NF-κB phosphorylation with antioxidant supplementation (Petersen, McKenna et al. 2011). These conflicting results on NF-κB activation highlight the complexity of exercise-induced redox sensitive signalling pathways. Whilst transient activation of the NF-κB pathway is required to regulate physiological adaptation, chronic increases in NF-κB activation have been associated with unwanted skeletal muscle atrophy and insulin resistance (Kramer and Goodyear 2007). However, the amount of exercise-induced oxidative perturbation that leads to this chronic activation of NF-κB remains unclear. Further research is required to establish the effects of NAC supplementation on the NF-κB redox signalling pathway during a block of intense physical training in well-trained athletes.

Previous research has identified a delicate balance between training-induced oxidant production and antioxidant protection. During strenuous periods of physical training, considerable stress is placed upon the antioxidant defence system, which may be unable to prevent the occurrence of excessive exercise-induced oxidative damage and inflammation. For example, a 4 wk overtraining period was shown to induce a state of oxidative stress and impair duathlon performance in nine male triathletes (Palazzetti, Richard et al. 2003). Athletes undergoing heavy training periods may require increased antioxidant support via exogenous supplementation (Palazzetti, Rousseau et al. 2004). However, the excessive consumption of antioxidant compounds has also been demonstrated to have a negative effect on the up-regulation of redox mediated adaptive processes to training stimuli (Gomez-Cabrera, Borras et al. 2005). It is therefore important to determine an appropriate match between additional antioxidant requirements and increased training demands in order to promote exercise-induced physiological adaptations.

The aims of the current investigation were: to establish the ergogenic potential of oral NAC supplementation on athletic performance during a cycle ergometer race simulation in well-trained triathletes and; to examine the impact NAC supplementation on oxidative stress parameters, antioxidant capacity, physiological adaptation (measured via changes in NF-κB p65 activity) and blood-borne markers of inflammation during a block of intensive training. It was anticipated that the results of the current investigation would demonstrate that whilst

antioxidant supplementation may improve performance in a specific bout of exercise, prolonged supplementation may hamper the adaptive processes in athletes.

Methods

Participants

Ten well trained male triathletes volunteered to participate in the investigation (age 23.6 ± 3.2 y, height 179.8 ± 4.4 cm, body mass 70.5 ± 7.2 kg, $\dot{V}O_2$peak 63.3 ± 4.8 ml/kg/min). The athletes regularly completed 15-25 h of swimming, cycling and running each week. Prior to the study, participants were informed of the testing requirements and potential risks involved in the study and gave written and verbal consent. The study was approved by the University of Technology, Sydney (UTS) Human Ethics Committee (Trial no 2010/0254 HREC 2009/164) and the use of oral NAC was approved by the Therapeutic Goods Administration of Australia.

Experimental design

The study used a double blind randomised placebo-controlled crossover design to investigate the effects of NAC supplementation on cycling performance and adaptation to physical training. In the 2 wk prior to the data collection period, participants were familiarised with the cycle ergometer race simulation (Vaile, Halson et al. 2008) and completed a cycling power profile test to determine $\dot{V}O_2$peak (Quod, Martin et al. 2010). All cycle tests throughout the study were performed on an SRM ergometer (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) under standardised laboratory conditions. On day 1 of the investigation, baseline venous blood and urine samples were taken and a pre-supplementation cycle ergometer race simulation was completed. Participants then began a 9 d supplementation period of either NAC or a placebo. On day 9 of the study, venous blood and urine samples were collected prior to, immediately following, 2 h and 24 h following the post-supplementation cycle ergometer race simulation. Time course changes were assessed in plasma for total antioxidant capacity (TAC), ferric reducing ability of plasma (FRAP), reduced glutathione (GSH) to oxidised glutathione (GSSG) ratio (GSH:GSSG), thiobarbituric acid-reactive substances (TBARS), IL-6 and MCP-1. In addition, changes in NF-κB within mononuclear cell extracts and urinary 15-isoprostane F_{2t} concentration (F_2-isoprostane) were also assessed at the same time points. Following a 3 wk washout period, the experimental

procedure was repeated with the remaining supplement (for schematic view of experimental design see Figure 6.1).

Supplementation

Participants refrained from consuming additional antioxidant supplements for at least 1 month prior to, and throughout the investigation. Participants were randomly assigned in a double blind manner to receive either 1200 mg per day of NAC (2 x 600 mg capsules) or 700 mg per day of a placebo (lactose; 2 x 350 mg capsules). Capsules for both NAC and placebo were identical in size and appearance. An additional dose of NAC or placebo was also consumed 2 h prior to the post-supplementation cycle ergometer session. Participants completed 4 d food records during each testing period to ensure a similar dietary intake. The food records were analysed using Foodworks software (version 6, 2009, Xyris, Qld, Australia). Participants were contacted on a daily basis during the supplementation period to ensure the supplements had been taken.

Cycle ergometer race simulation

Following familiarisation, participants performed the cycle ergometer session (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) pre- and post- each supplementation period. The exercise was similar to the 105 min fatigue-inducing cycle protocol described previously (Vaile, Halson et al. 2008) (Table 6.1). This protocol was chosen to mimic the demands of a cycling race and allow for changes in both anaerobic and aerobic performance capacity to be assessed. The typical error of this protocol has been reported as 17.1 W and 2.1% (Vaile, Halson et al. 2008). Participants were instructed to perform each sprint maximally and to complete as much work as possible during each steady state time trial effort. Heart rate (HR) (Suunto dual belt, Vantaa, Finland) and power data were recorded every second. Each session was analysed using the SRM software program (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) to determine average power output, work (kJ) and HR for each effort.
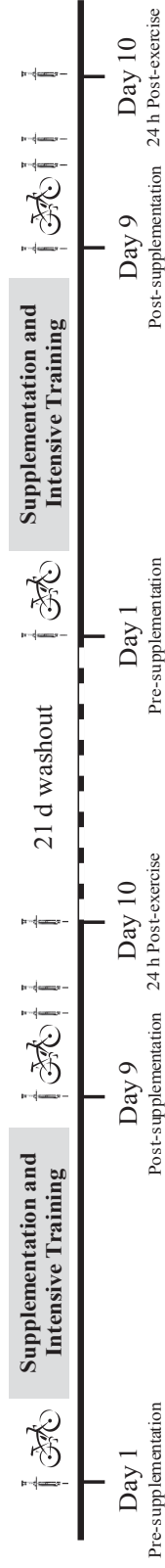
Figure 6.1: Schematic diagram of the experimental design. Cycle ergometer race simulation, Venous blood and urinary samples.

Table 6.1: Cycle ergometer race simulation protocol.

Cycle Ergometer Race Simulation	
Warm-up	10 min with 3 x 3 s efforts
Set 1	12 x 5 s efforts with 30 s rest
Rest	*5 min easy*
Set 2	12 x 5 s efforts with 15 s rest
Rest	*5 min easy*
Set 3	12 x 5 s efforts with 5 s rest
Rest	*5 min easy*
Time Trial 1	2 min
Rest	*5 min easy*
Set 4	6 x 10 s efforts with 60 s rest
Rest	*5 min easy*
Set 5	6 x 10 s efforts with 30 s rest
Rest	*5 min easy*
Set 6	6 x 10 s efforts with 10 s rest
Rest	*5 min easy*
Time Trial 2	2 min
Rest	*5 min easy*
Set 7	4 x 15 s efforts with 90 s rest
Rest	*5 min easy*
Set 8	4 x 15 s efforts with 45 s rest
Rest	*5 min easy*
Set 9	4 x 15 s efforts with 15 s rest
Rest	*5 min easy*
Time Trial 3	5 min
Rest	*5 min easy*

Physical training and Daily Analysis of Life Demands of Athletes

Physical training was recorded during the experimental period using the session-RPE method which is calculated as the product of training duration (min) and the mean training intensity (rating of perceived exertion CR-10) (Foster, Florhaug et al. 2001). The participants were asked to replicate a similar training program during each experimental period, which was supervised by the investigators or each athlete's respective coach. Participants also completed the Daily Analyses of Life Demands for Athletes (DALDA) questionnaire at the same time each day to assess general stress levels and to determine stress-reaction symptoms of the participants (Rushall 1990). An elevated number of 'worse than normal' responses were used to determine when an athlete was in a state of stress.

Biochemistry measures

Venous blood and urine samples were collected at five time points during each intervention. On day 1, a resting baseline sample was collected. Then following the 9 d supplementation period additional samples were collected at rest, immediately following, 2 h and 24 h following the post-supplementation cycle ergometer race stimulation. Blood samples were collected into 2 x 6 ml EDTA lined vacutainer tubes and 1 x 8 ml cell preparation tube with sodium heparin (Becton Dickson, Franklin Lakes, NJ), which were centrifuged at 3000 g for 15 min immediately after collection at 4°C. Plasma, supernant containing mononuclear cells and urine were transferred into 500 µL aliquots, frozen in liquid nitrogen and stored at -80°C until assayed.

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the plasma concentration of MCP-1 (Quantikine Immunoassay, R&D Systems, Minneapolis, USA) (intra-assay CV: 7.4%), IL-6 (Quantikine HS ELISA, R&D Systems, Minneapolis, USA) (intra-assay CV: 7.6%), TAC (Cayman Chemical Company, Ann Arbor, MI, USA) (intra-assay CV: 5.4%) and TBARS (Oxford Biomedical Research, MI, USA) (intra-assay CV: 5.7%). NF-κB p65 DNA binding activity was measured in nuclear extracts obtained from peripheral mononuclear cells using a nuclear extraction kit (Cayman Chemical Company, Ann Arbor, MI, USA) and analysed with a NF-κB p65 Transcription Factor Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) (intra-assay CV: 6.3%). An ELISA kit was also used to measure the urinary F_2Isoprostane concentration (Oxford Biomedical Research, MI, USA) which was normalised against urinary creatinine concentration (Oxford Biomedical Research, MI, USA). All assay kits were completed according to the manufacturer's instructions on a Bio-Rad plate reader (Hercules, CA, USA).

GSH and GSSG were measured in plasma treated with 3% 5-sulfosalicyclic acid and analysed enzymatically according to the method of Baker et al. (Baker, Cerniglia et al. 1990). Briefly, GSH concentration was determined by assessing the rate of enzymatic recycling by means of 5,5'-dithiobis 2-nitro-benzoic acid, glutathione reductase and NADPH at 405 nm on a Bio-Rad plate reader (Hercules, CA) (intra-assay CV: 7.3%). GSSG was measured using the same protocol after the removal of GSH from the plasma through the addition of 2-vinylpyridine and triethanolamine to each sample (intra-assay CV: 3.9%). Standard curves from stock solutions of GSH and GSSG were created using serial dilutions with 3% 5-

sulfosalicyclic acid. The ratio of GSH to GSSG (GSH:GSSG) was calculated as [(GSH − (2•GSSG)]/GSSG.

The FRAP assay was conducted using a modification of the original method described by Benzie and Strain (Benzie and Strain 1996). Plasma samples and FRAP reagent (300 mM acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine, 40 mM hydrochloric acid and 20 mM Iron (III) chloride hexahydrate) were added to a microtitre plate and incubated for 4 min at 37°C. Absorbance was measured on a Bio-Rad plate reader (Hercules, CA) at 593 nm and compared to an Iron (II) sulphate standard curve (intra-assay CV: 2.4%). All reagents were purchased from Sigma-Aldrich® (St. Louis, MO).

Statistical analyses

Prior to using parametric statistical procedures, the assumption of sphericity was verified. Statistica 8.0 software (StatSoft Inc. Tulsa, USA) was used for all calculations. An a priori sample analysis revealed 8 pairs of subjects is the minimum required in a matched pair design to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05 (G*Power version 3.1.3, Universität Kiel, Germany). Changes in biochemical parameters were analysed using a factorial two-way analysis of variance (ANOVA) by condition (NAC and placebo supplementation) and time (pre-supplementation and post-supplementation pre-exercise, immediately post-exercise, 2 h and 24 h post-exercise). Performance variables during the cycle ergometer test were also analysed with a factorial ANOVA by condition (NAC and placebo supplementation) and time (pre-supplementation and post-supplementation). If significant main effects were observed, a Scheffe *post hoc* analysis was used to locate the source of the differences. The magnitude of partial eta squared effects were interpreted as trivial (< 0.001), small (0.001 − 0.089), moderate (0.09 − 0.25) and large (> 0.25), respectively (Cohen 1988). A paired samples t-test was used to determine differences in training load, DALDA and nutrient intake between the experimental periods. Statistical significance was accepted at $P < 0.05$. Data are presented as mean ± SD.

Results

Eight of the 10 participants completed both the NAC and placebo trials. One participant withdrew due to injury, the other due to illness during the second supplementation period. Data from these participants was not included in the analysis. No adverse events or side effects were reported by participants for either the NAC or the placebo supplements. The participants completed a similar amount of training duration (NAC 20.1 ± 3.7 h; placebo 19.6 ± 2.8 h, P = 0.72) and intensity (RPE, NAC 5.9 ± 1.4 ; placebo 6.3 ± 0.7, P = 0.74). A significant increase (P = 0.05) was observed in the number of 'worse than normal' responses reported during the placebo period (57.0 ± 54.3) than when the NAC supplement (25.0 ± 19.7) was consumed. No significant difference was observed in the macro- or micro-nutrient intake of the participants during the two supplementation periods.

Repeated sprint performance during the cycle ergometer race simulation was significantly improved (P < 0.001, η^2_p = 0.03) during the 5 s, 10 s and 15 s efforts when athletes consumed NAC as opposed to the placebo (Figure 6.2). No change was observed in mean heart rate, total work or mean power during each steady state time trial effort between the NAC or placebo trials (Table 6.2). No significant differences were shown in lactate or RPE measures taken throughout the cycle sessions (data not shown).
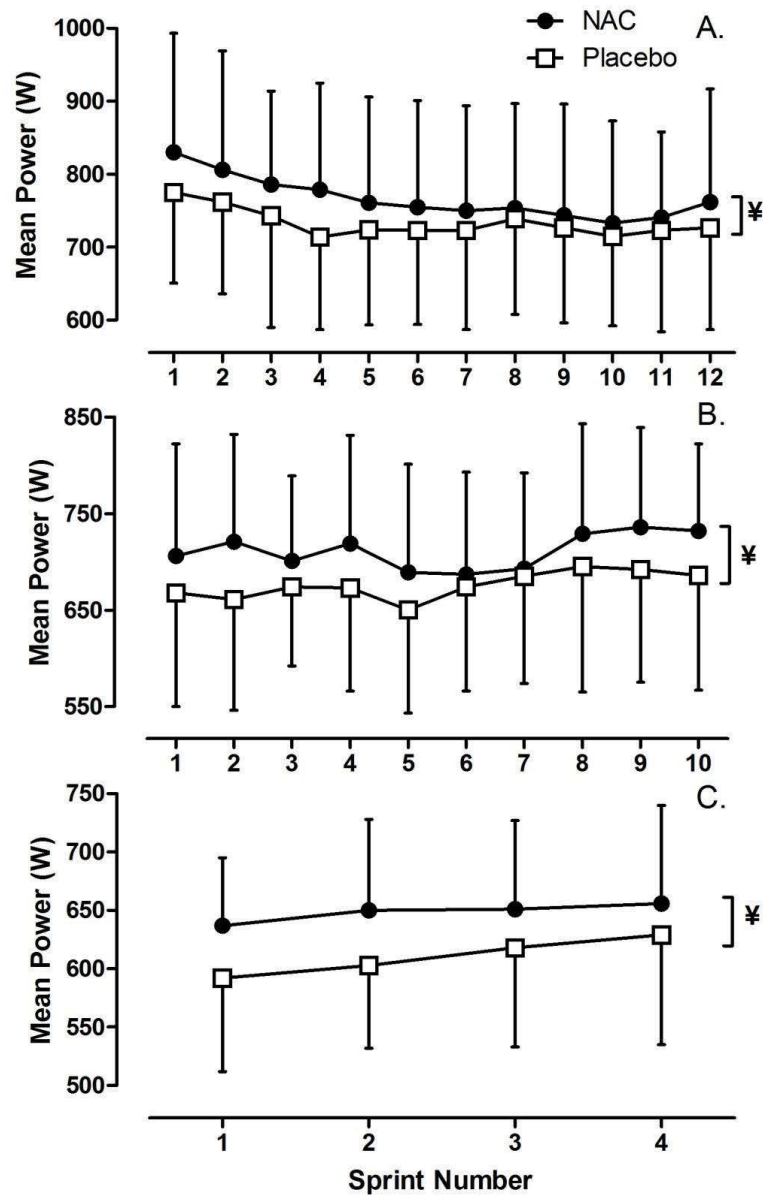
Figure 6.2: Changes in A. 5 s, B. 10 s and C. 15 s mean power during the post-supplementation cycle ergometer race simulation (mean ± SD). ¥Significant main effect for condition ($P < 0.001$).

Table 6.2: Time trial performance parameters before and after supplementation with both NAC and placebo (mean ± SD).

		Time Trial 1		Time Trial 2		Time Trial 3	
		Pre	Post	Pre	Post	Pre	Post
Mean Power (W)	Placebo	405 ± 31	402 ± 41	375 ± 46	384 ± 34	327 ± 36	338 ± 39
	NAC	390 ± 46	399 ± 38	392 ± 42	399 ± 46	327 ± 40	345 ± 40
Mean Heart Rate (bpm)	Placebo	166 ± 9	166 ± 10	161 ± 9	162 ± 7	165 ± 9	167 ± 8
	NAC	161 ± 7	158 ± 9	161 ± 8	157 ± 8	164 ± 9	163 ± 10
Total Work (kJ)	Placebo	44 ± 7	45 ± 5	44 ± 7	45 ± 5	98 ± 11	101 ± 11
	NAC	47 ± 5	47 ± 6	47 ± 5	47 ± 6	97 ± 12	102 ± 12

NAC supplementation increased plasma antioxidant capacity as observed via a significant interaction effect in plasma TAC (P = 0.005, η^2_p = 0.19) (Figure 6.3b) and a greater plasma GSH concentration by condition (NAC 13.16 ± 0.98 µM, placebo 10.19 ± 0.98 µM, P = 0.036, η^2_p = 0.06, Figure 6.4b). However, this finding was not reflected in plasma FRAP measures. A significant effect was observed in plasma FRAP for time with an overall increase from pre- to post-exercise (P = 0.016, η^2_p = 0.16) (Figure 6.3a) without an interaction or condition main effect.
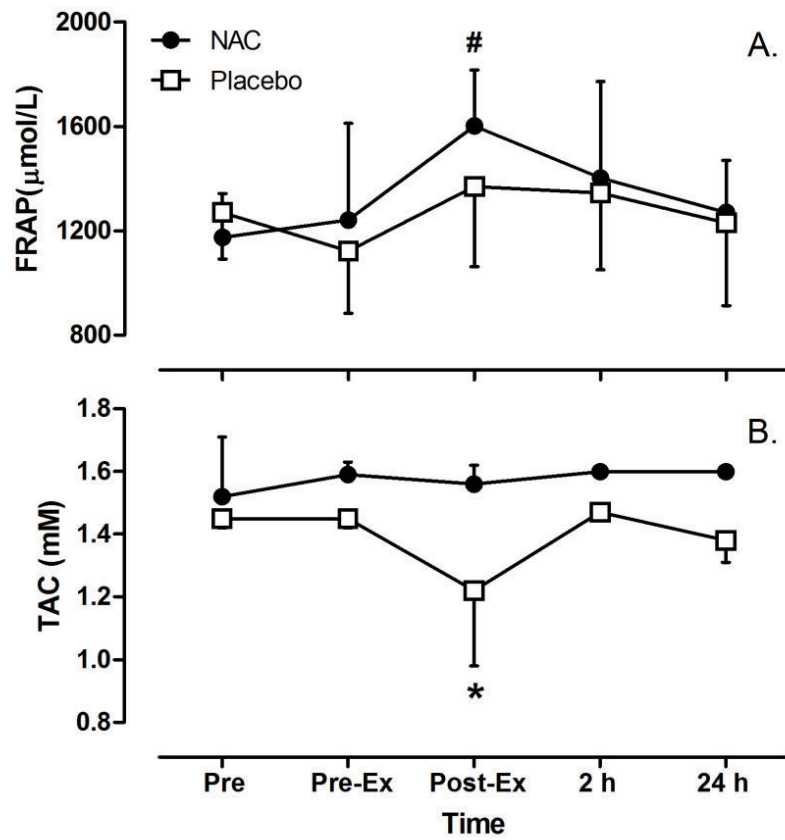
Figure 6.3: Time course of changes in A. ferric reducing ability of plasma (FRAP) and B. plasma total antioxidant capacity (TAC). Pre (pre-supplementation and pre-exercise), Pre-ex (pre-exercise following supplementation with NAC and placebo), Post-ex (post-exercise), 2 h (2 h post-exercise) and 24 h (24 h post-exercise) (mean ± SD). *Significant interaction effect ($P < 0.01$). #Significant effect for time ($P < 0.05$).

Figure 6.4: Time course of changes in A. plasma reduced glutathione to oxidised glutathione ratio (GSH:GSSG), B. plasma GSH and C. plasma GSSG. Pre (pre-supplementation), Pre-ex (pre-exercise following supplementation with NAC and placebo), Post-ex (post-exercise), 2 h (2 h post-exercise) and 24 h (24 h post-exercise) (mean ± SD). ¥Significant effect for condition ($P < 0.05$).

A reduction in plasma and urinary markers of oxidative damage following the cycle ergometer simulation was demonstrated with NAC supplementation. A significant interaction effect was observed in plasma TBARS ($P = 0.002$, $\eta^2_p = 0.22$) (Figure 6.5a) with post-hoc analysis revealing a significant reduction in plasma TBARS immediately post-exercise in the NAC supplementation intervention. Post-hoc analysis showed a significantly greater urinary F_2Isoprostane concentration both prior to and immediately following the cycle ergometer race simulation when the placebo was consumed ($P = 0.010$, $\eta^2_p = 0.431$) (Figure 6.5b). No change was observed in the plasma GSH:GSSG ratio for time or condition (Figure 6.4a).
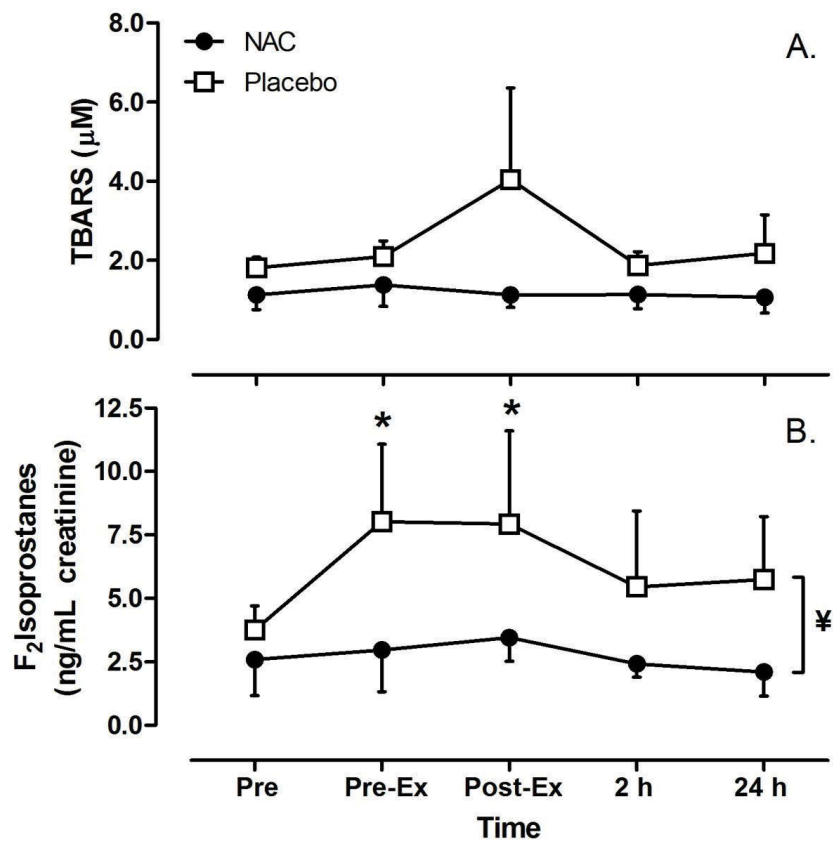
Figure 6.5: Time course of changes in A. plasma thiobarbituric acid-reactive substances (TBARS) and B. urinary 15-isoprostane F_{2t}-concentration (F_2-isoprostane). Pre (pre-supplementation), Pre-ex (pre-exercise following supplementation with NAC and placebo), Post-ex (post-exercise), 2 h (2 h post-exercise) and 24 h (24 h post-exercise) (mean ± SD). *Significant interaction effect ($P < 0.01$). ¥Significant main effect for condition ($P < 0.01$).
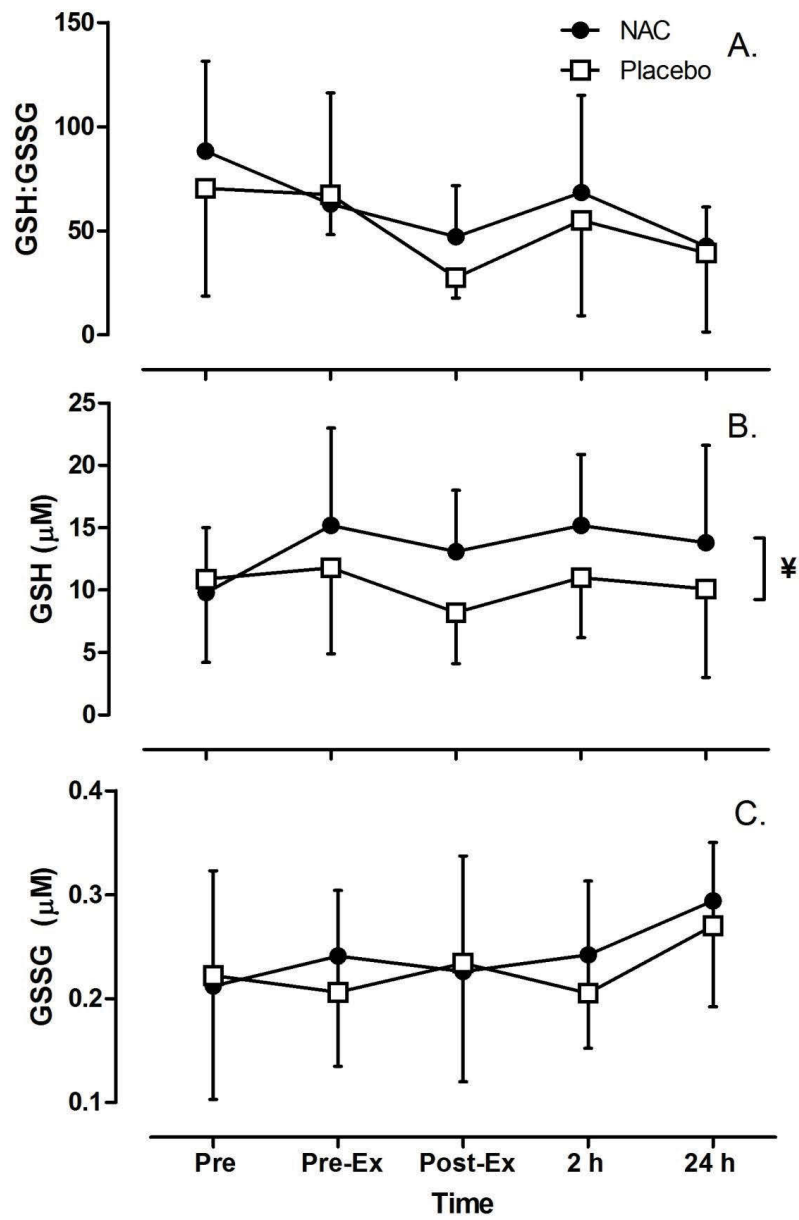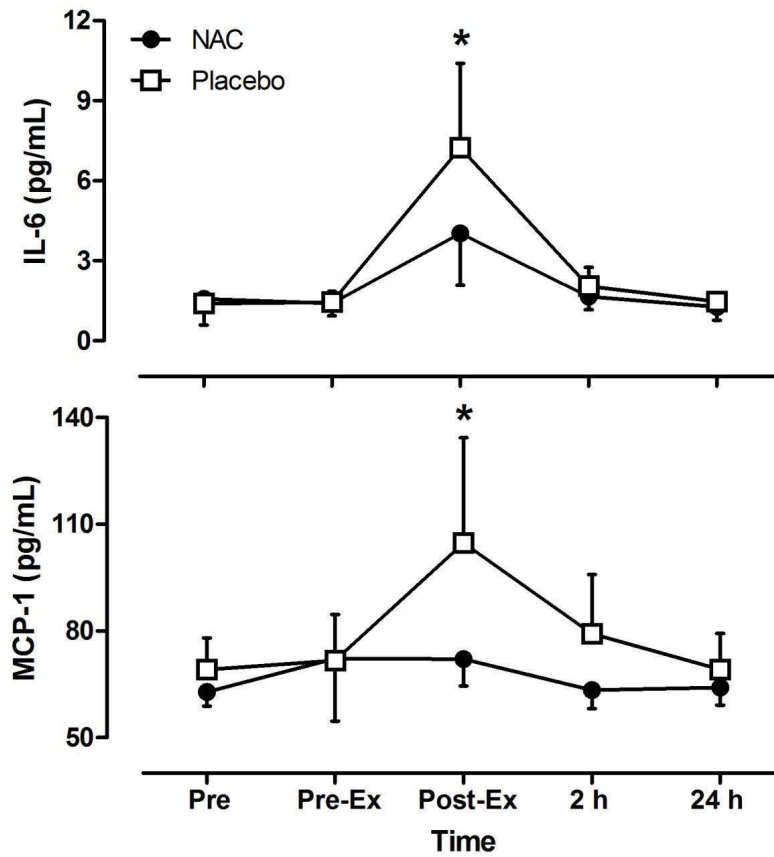
Figure 6.6: Time course of changes in A. plasma interlukin-6 (IL-6) and B. plasma monocyte chemoattractant protein-1 (MCP-1). Pre (pre-supplementation), Pre-ex (pre-exercise following supplementation with NAC and placebo), Post-ex (post-exercise), 2 h (2 h post-exercise) and 24 h (24 h post-exercise) (mean ± SD). *Significant interaction effect ($P < 0.01$).

NAC supplementation significantly blunted the exercise-induced increases in plasma concentration of both IL-6 ($P = 0.002$, $\eta^2_p = 0.22$) and MCP-1 ($P = 0.012$, $\eta^2_p = 0.17$) (Figure 6.6). A significant time ($P = 0.002$, $\eta^2_p = 0.21$) and condition effect ($P < 0.001$, $\eta^2_p = 0.21$) was observed in NF-κB activation with a greater increase in activity at 2 h post exercise when NAC supplementation was consumed as opposed to the placebo (Figure 6.7).
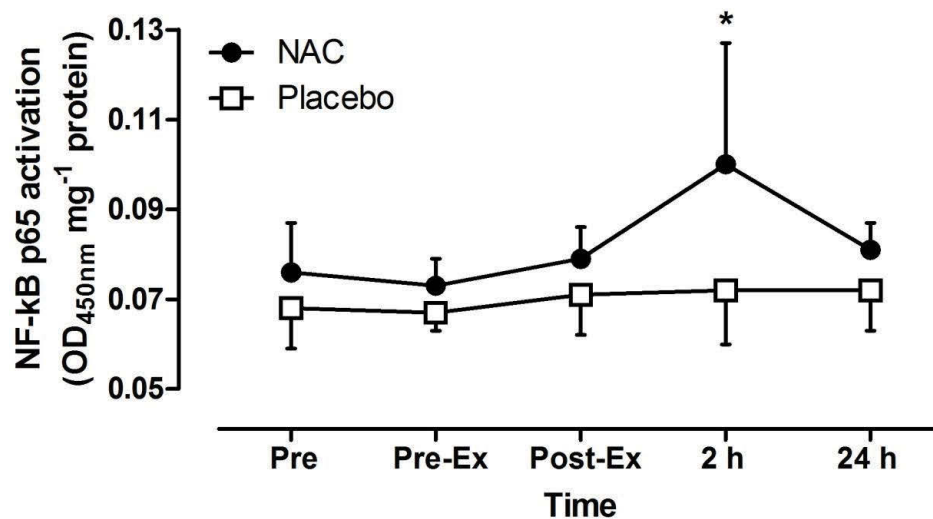
Figure 6.7: Time course of changes in peripheral mononuclear cell extract nuclear factor-kappaB (NF-κB).   Pre (pre-supplementation), Pre-ex (pre-exercise following supplementation with NAC and placebo), Post-ex (post-exercise), 2 h (2 h post-exercise) and 24 h (24 h post-exercise) (mean ± SD).   *Significant interaction effect ($P < 0.01$).

Discussion

The purpose of this study was to examine the ergogenic potential of oral NAC supplementation on race specific cycling performance and associated exercised-induced changes within the oxidative and inflammatory pathways in highly-trained athletes during heavy training. The findings provide novel evidence that oral NAC supplementation induces a significant improvement in repeated cycle (anaerobic) sprint performance compared to a placebo in well-trained triathletes. This supports the findings of previous laboratory-based research that has identified the ability of NAC to promote fatigue resistance during strenuous exercise (Reid, Stokic et al. 1994; Medved, Brown et al. 2004; McKenna, Medved et al. 2006). Similar increases in performance have also recently been reported during repeated high-intensity intermittent shuttle runs in recreationally trained men following a 6 d loading period with a 50 mg·kg^{-1} dose of NAC (Cobley, McGlory et al. 2011). Collectively, these studies demonstrate that the usefulness of NAC supplementation extends past the laboratory, and can improve athletic performance in a practical sporting environment.

Whilst the mechanisms underlying the sprint performance changes during exercise were not directly measured in this study, other research has identified potential physiological factors, which may explain the ergogenic effect of NAC (Reid, Stokic et al. 1994; McKenna, Medved et al. 2006). It has been suggested that NAC acts to minimise ROS accumulation within contractile tissue, which can assist in the maintenance of force production during fatiguing skeletal muscle contractions (Reid, Stokic et al. 1994). Specifically, the elevated antioxidant capacity with NAC may reduce ROS interference within the sodium, potassium (Na^+,K^+)-pump activity (McKenna, Medved et al. 2006) and calcium regulation (Andrade, Reid et al. 2001) during the excitation contraction coupling process. Indeed, the observed performance and physiological benefits with NAC supplementation in the current study may reflect an enhanced ability to maintain an optimal redox balance during exercise.

When participants were supplemented with NAC, plasma TAC concentration remained elevated post-exercise compared to the significant reduction observed in plasma TAC in the placebo condition. In addition, exercise-induced increases in markers of oxidative damage were completely blunted in the NAC condition. This finding is comparable with other investigations, whereby supplementation with antioxidants did not increase basal antioxidant capacity, but did effectively counterbalance the oxidative insult during exercise (Margaritis, Palazzetti et al. 2003; Teixeira, Valente et al. 2009). Similarly, the dosage of NAC used in the current study may have been insufficient to alter plasma glutathione status. For instance, a recent investigation demonstrated that oral NAC doses of 1200 mg did not affect the glutathione-based thiol concentration in human plasma (Ferreira, Campbell et al. 2011). Nonetheless, it is apparent that NAC can exert a performance benefit irrespective of changes in plasma glutathione metabolism (Medved, Brown et al. 2004; Matuszczak, Farid et al. 2005). The results of the present investigation suggest that NAC supplementation can reduce exercise-induced redox perturbations and improve exercise performance. However, improvements in performance with NAC supplementation have not been consistently shown (Medved, Brown et al. 2003; Medved, Brown et al. 2004; Bailey, Winyard et al. 2011).

In the current investigation, despite a significant improvement in repeat cycle sprint performance, no change in steady state time trial (2 min or 5 min) performance was observed following the NAC supplementation period. These observations are in contrast to the current consensus that NAC is more effective in enhancing sub-maximal exercise performance (Matuszczak, Farid et al. 2005) rather than anaerobic activity (Medved, Brown

et al. 2003; Corn and Barstow 2011). Experimental evidence on the effects of NAC in isolated muscle fibres has previously demonstrated an inhibition of low-frequency contractile fatigue without a concomitant reduction in fatigue during high-frequency stimulation (Reid, Stokic et al. 1994). Similarly, in whole body exercise, NAC infusion failed to improve repeat sprint cycling performance (4 x 45 s maximal efforts) in eight untrained men (Medved, Brown et al. 2003). Alternatively, in a separate study by the same investigators, a significantly improved cycle time to exhaustion at 92% $\dot{V}O_2$peak with NAC infusion was reported in eight well-trained men (Medved, Brown et al. 2004). These equivocal results regarding the ergogenic effect of NAC, may be in part due to the training status of the participants, differing supplementation protocols and the varied metabolic demands of each exercise protocol. Further speculation on the underlying contributors to this observed difference in improvements during repeat sprint *vs.* short duration steady state time trial performance is beyond the scope of this investigation. Future research is therefore required to fully elucidate the respective dosage and mode of exercise whereby NAC potentiates an ergogenic effect for well-trained endurance athletes.

To the authors' knowledge, this was the first study to investigate the effects of a short-term (9 d) oral NAC supplementation protocol on the physiological responses to training stimuli, using a crossover experimental design. In comparison with previous studies investigating NAC supplementation, the present study administered a comparatively low dose of NAC (1200 mg·d^{-1}). The supplementation protocol was chosen in a successful attempt to prevent the occurrence of unwanted side effects from consuming oral NAC capsules such as nausea, diarrhoea, flatulence and sleepiness (Ferreira, Campbell et al. 2011). This lack of adverse reactions improves the efficacy of incorporating NAC supplementation into an athlete's daily training environment.

Another potential concern in the current investigation was that the consumption of NAC over a 9 d loading period would negate beneficial physiological adaptive responses from occurring. This was based on the findings of several prior studies, which have demonstrated the ability of antioxidant compounds to interfere with the gene transcription pathways and ameliorate redox regulated adaptations to exercise in human participants (Gomez-Cabrera, Martinez et al. 2006; Petersen, McKenna et al. 2011). However, the results of the present study provide contrary evidence that suggests that NAC supplementation promoted physiological adaption following the cycle race simulation protocol. This was indicated by the significantly greater 2 h post-exercise increase in NF-κB activation with NAC compared to

the placebo supplementation. NAC supplementation also appeared to enhance the participant's ability to tolerate physical training. When consuming NAC, participants reported a significantly reduced number of 'worse than normal responses' in the DALDA and reduced levels of basal oxidative damage as measured in urinary F_2Isoprostane compared to the placebo condition. In addition, NAC blunted exercise-induced increases in the cytokines IL-6 and MCP-1 following the cycle ergometer race simulation. These results are in agreement with previous research that has demonstrated no negative effects of antioxidant supplementation on physiological adaptive processes (Chang, Hu et al. 2010; Yfanti, Akerstrom et al. 2010) and a greater ability to cope with periods of demanding physical training (Palazzetti, Rousseau et al. 2004; Zoppi, Hohl et al. 2006).

There are a myriad of physiological, environmental, biomechanical, psychological and lifestyle factors that contribute to an athlete's ability to complete physical training loads and produce optimal performance. The present findings show that short-term supplementation with the antioxidant NAC improves repeated sprint cycling performance and promotes the maintenance of an optimal redox balance during exercise. In addition, the relatively low dose (i.e. 1200 mg/d) of NAC did not appear to incur a negative impact on the adaptive responses to exercise. Instead, NAC supplementation enhanced the oxidant-antioxidant balance during exercise and provided a more conducive environment for muscle contraction and adaptation to occur in well-trained triathletes undergoing a period of intensive physical training. However, a 9 d supplementation period may not be of sufficient duration to significantly impact training-induced physiological adaption. Further research is required to investigate the impact of prolonged NAC loading periods on performance and adaptive responses to exercise stimuli within the daily training environment.

CHAPTER SEVEN

General Discussion

1. Main Findings

Exercise induces a systemic stress response that is exacerbated during intensified physical training. However, the training stimulus that promotes adaptive or maladaptive responses within biological systems remains unclear. Therefore, a series of applied research investigations were conducted to develop a greater understanding of the relationship between the oxidative, inflammatory and neuroendocrinological systems in well-trained athletes during intensive physical training.

Effect of intensified training on the oxidative, inflammatory and neuroendocrinological systems and performance

The physiological responses to exercise are influenced by the interplay between oxidative, inflammatory and neuroendocrinological systems. This thesis provides significant insight into the interactions within these respective systems via the measurement of exercise-induced changes in plasma and urinary markers. The common finding of Study 2 and Study 3 was that exercise induced disturbances in reduction-oxidation (redox) homeostasis associated with acute fatigue may be an underlying factor in performance decrements following intensified physical training. When a greater training stress was applied, a decreased performance was coupled with a sustained elevation in xanthine oxidase (XO) in Study 2 and increases in oxidative damage [thiobarbituric acid-reactive substances (TBARS) and 15-isoprostane F_{2t} concentration (F_2Isoprostane)] during Study 3. In addition, following N-acetylcysteine (NAC) supplementation in Study 3, the elevated antioxidant capacity was associated with a reduced inflammatory reaction [interlukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1)] and an improved performance. Interestingly, irrespective of the preceding training load [intensive (HIGH) or reduced (LOW)], a rise in post-exercise plasma IL-6 and MCP-1 was observed in Study 2. The comparable amount of inflammation after both HIGH and LOW physical training could reflect the demanding nature of the high intensity intermittent running protocol. Indeed, MCP-1 release was greater in the LOW condition in accordance with the increased exercise intensity during the running protocol. It may also provide added evidence that NAC supplementation is a potent modulator of redox homeostasis and by reducing the rate of oxidation, NAC can attenuate exercise-induced inflammatory responses.

Increases in training load had no effect on the plasma concentration of either growth hormone (GH) or the thyroid hormones in Study 2. Consequently, neuroendocrinological measures were not taken in Study 3. Instead, the transcription factor, nuclear factor - kappaB (NF-κB) was assessed to provide an indication of adaptation via redox sensitive signalling pathways. In contrast to the initial hypothesis that NAC would blunt redox mediated adaptation, an increased NF-κB activity was observed in the NAC condition. This increased NF-κB phosphorylation, suggests that antioxidant supplementation during periods of stressful physical training may act to preserve redox balance and promote an adaptive state. The collective results showed that when redox homeostasis was disturbed by acute training fatigue, increases in oxidative damage and inflammation occurred without any observable effect on plasma hormonal parameters. Taken together, these findings demonstrate that acute intensified periods of physical training place considerable stress upon the oxidative and inflammatory systems. If intensive physical training is continued, a dysregulation in the oxidative and inflammatory systems may contribute to exercise maladaptation and possibly disturbances in the neuroendocrinological system.

Impact of antioxidant supplementation on physical training and performance

Many athletes habitually consume additional antioxidant compounds to supplement their dietary intake from whole foods. Whilst, this practice can assist in the reduction of exercise-induced oxidative damage, it may also blunt the up-regulation of endogenous antioxidant enzymes and other redox sensitive signalling pathways (Ristow, Zarse et al. 2009). The results of Study 1 demonstrated that, according to the Australian dietary guidelines, the antioxidant intake (vitamin A, vitamin C and vitamin E) of well-trained athletes was sufficient. Based on these findings, it is apparent that an adequate antioxidant intake can be achieved via dietary sources and exogenous supplementation is not required. However, intensified physical training may place considerable demand on the antioxidant defence system. For example, Study 3 showed that without additional antioxidant support from NAC, participants displayed a reduced antioxidant capacity and incurred a greater amount of oxidative damage / inflammation. This observed redox imbalance suggests that athletes may benefit from antioxidant supplementation during periods of increased exercise-stress. In addition, NAC may provide further advantage to athletes, as indicated by the observed performance improvements in Study 3. The collective findings of the thesis provide novel evidence which indicates that short-term supplementation with NAC can assist athletes tolerate higher training loads and can exert an ergogenic effect during exercise.

Proposed model of interactions between the oxidant-antioxidant balance during exercise-stress in fatigued and non-fatigued states

An imbalance between exercise-stress and recovery during intensified physical training periods can increase fatigue and impair adaptation. The findings reported in this thesis suggest that an altered physiologic response occurs in the oxidative and inflammatory systems when athletes perform exercise in a fatigued *vs.* non-fatigued state. When strenuous exercise is performed in a non-fatigued state, additional antioxidant supplementation, has a minimal effect on exercise performance and may impair redox signalling cascades (Ristow, Zarse et al. 2009). However, the findings of Study 3 showed that NAC supplementation allowed triathletes to better tolerate intensive physical training, increased repeat sprint power output and augmented NF-κB activity. Figure 7.1 presents the hypothesis that when athletes can adequately tolerate a training stimulus, exogenous antioxidant supplementation will provide no additive benefit to performance and may impair adaptation. In contrast, when athletes are in a fatigued state (i.e. intensified training, strenuous exercise, illness, altitude exposure), antioxidant supplementation can promote adaptation and improve performance. This is supported by previous studies that have demonstrated a greater ergogenic effect of NAC in pre-fatigued skeletal muscle (Reid, Stokic et al. 1994; Cobley, McGlory et al. 2011).

Cellular repair and regeneration of skeletal muscle may be attenuated if the inflammatory reaction is suppressed via antioxidant supplementation (Tidball 2005; Close, Ashton et al. 2006). However, successive bouts of strenuous exercise can promote a state of chronic inflammation that may contribute to increased cellular degradation and interference in physiologic function (Smith 2004). Antioxidant supplementation during intensified training periods may help buffer the additional oxidants released as part of the inflammatory cascade. This lesser inflammatory response may then reduce susceptibility to the development of chronic inflammation and allow athletes to continue to complete demanding physical training loads. It is likely that, in Study 3, NAC assisted in the regulation of the inflammatory response and reduced excessive exercise-induced oxidative damage. This hypothesis shows that it is important to match an athlete's training and competitive demands with an appropriate level of antioxidant supplementation. Further research in well-trained athletes is required to test this hypothesis in a the daily training environment.
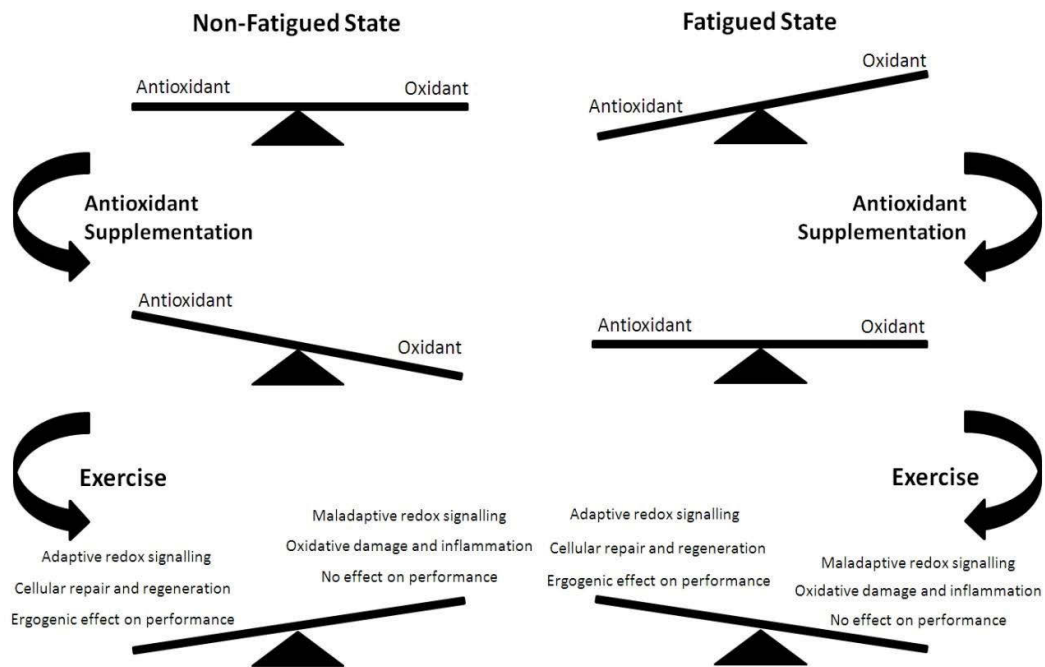
Figure 7.1: Effect of exogenous antioxidant supplementation dependent on an athlete's level of fatigue.

2. Limitations

Due to the applied approach taken in this thesis, the findings are limited to observations in plasma and urine, as opposed to at a skeletal muscle level. It was impractical to complete serial muscle biopsies on well-trained athletes. However, further information regarding exercise-induced up-regulation of redox sensitive target genes via the NF-κB and mitogen activated protein kinase (MAPK) pathways would have allowed a more in-depth understanding of the observed responses in the current investigations. Nonetheless, by assessing changes in plasma and urine, the present results can be used to monitor fatigue and adaptive state in an athlete's daily training environment. The small sample sizes due to the subject delimitation to well-trained athletes and monetary funding is a limitation that reduces statistical power. Whilst the series of investigations examined different sporting populations (i.e. swimming, triathlon and team sport), the commonality of intensified training and the level of athletes in their respective sport, allows for collective interpretation of the data. Another limitation of the current thesis, is the lack of dietary information within Australian food tables concerning antioxidant compounds. The tables do not provide

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information on the nutritional content of antioxidant compounds such as polyphenols, glutathione, coenzyme Q10, lycopenes and selenium, which does not allow the accurate assessment of total antioxidant intake.

3. Practical Applications

The findings of the thesis have identified practical recommendations regarding physical training monitoring, exercise prescription and antioxidant supplementation, which can be applied in a sporting setting;

- Negative physiologic responses (i.e. elevated oxidative damage, increased inflammation) are exacerbated in athletes who are in a fatigued state. Therefore, following the completion of demanding physical training sessions, it is likely that fatigued athletes will require extended recovery periods.
- Athletes in a fatigued state have a reduced capacity to perform repeated high-intensity efforts. Consequently, to gain the desired adaptive response, anaerobic based training sessions should be completed in a non-fatigued state.
- Monitoring changes in markers of oxidative damage and / or antioxidant capacity may provide insight into the global effect of physical training load and resultant fatigue state in athletes.
- Changes in post-exercise plasma measures provide a more accurate indication of physical training tolerance in athletes than resting plasma measures.
- If consuming a well-balanced diet, athletes in a non-fatigued state, do not require additional antioxidant supplementation.
- NAC antioxidant supplementation should be reserved for use during periods of increased exercise-stress or as an ergogenic aid during competitive performance.

CHAPTER EIGHT

Summary and Recommendations

1. Thesis Summary

Physical training places considerable stress on biological systems, which is a necessary trigger for physiological adaptation. It has been demonstrated that alterations in the reduction-oxidation (redox) balance may be an underlying mediator of exercise-induced adaptive processes and can have a direct influence on muscle contractile force. However, it has proved difficult to extrapolate findings from animal and *in situ* based experimental designs to be relevant within a practical sports setting. Previous research on exercise-induced perturbations within biological systems and antioxidant supplementation in athletes has produced equivocal results. This thesis aimed to further investigate global changes within the oxidative, inflammatory and neuroendocrinological responses to exercise using an applied research approach to establish; the nutritional antioxidant intake of athletes (Study 1); the impact of increased training load on oxidative damage, inflammation, hormonal disturbances and performance capacity (Study 2); the effect of antioxidant supplementation on exercise-induced changes in redox balance and inflammation on adaptive processes (Study 3); and the potential ergogenic effect of oral supplementation with the antioxidant N-acetylcysteine on cycling performance (Study 3). A summary of the findings from the series of investigations conducted as part of the thesis is shown in Table 8.1.

Table 8.1: Summary of the investigations conducted as part of the thesis.

Study (Number, Chapter, Title)	Subjects	Study Design	Supplement (Daily Dose)	Training Load	Performance Test	Findings
1 Chapter 3 Antioxidant intake of well-trained athletes during intensified physical training	23 well-trained athletes (m=21, f=2)	Observational design	no	Intensified	n/a	Athletes consumed above the Australian recommended daily intake for vitamin A, vitamin C and vitamin E.
2 Chapter 4 Effect of training load on simulated team sport match performance	8 male well-trained team sport athletes (n=8)	Cross-over design	no	Intensified and Low	High Intensity Intermittent Running Protocol	↓ running performance following 4 d intensified training was associated with ↑ plasma xanthine oxidase post-exercise.
2 Chapter 5 Evaluating the effect of acute changes in training load on select oxidative, inflammatory and endocrinological markers in team sport players	8 male well-trained team sport athletes (n=8)	Cross-over design	no	Intensified and Low	High Intensity Intermittent Running Protocol	↓ running performance after the 4 d intensified training. No difference between conditions were observed for pre- or post-exercise measures of plasma thyroid hormones, plasma GH or oxidative damage (urinary F_2-isoprostane).
3 Chapter 6 The effect of N-acetylcysteine on cycling performance following intensified training in well-trained triathletes: a double blind randomised placebo controlled study	8 male well-trained triathletes (n=8)	Double-blind placebo-controlled cross-over design	NAC (1200 mg/day)	Intensified	Cycle Ergometer Race Simulation	9 d NAC supplementation improved sprint cycling performance and post-exercise measures ↓ oxidative damage (urinary F_2-isoprostane, plasma TBARS), ↓ inflammatory (plasma IL-6, MCP-1), ↑ plasma TAS and ↑ peripheral mononuclear cell extract NF-κB activity.

F_2-isoprostane = 15-isoprostane F_{2t} concentration; **GH** = growth hormone; **IL-6** = interlukin-6; **NAC** = N-acetylcysteine; **NF-κB** = nuclear factor-kappaB; **MCP-1** = monocyte chemoattractant protein-1; **TAC** = total antioxidant capacity and **TBARS** = thiobarbituric acid-reactive substances

The findings of the current thesis contribute to the prior knowledge base on the dose-response relationship between redox balance and exercise stimuli in well-trained athletes. An acute block of intensified physical training was shown to increase oxidative damage / inflammation and induce decrements in anaerobic performance capacity. The results of the thesis suggest that the body responds differently to changes in redox disturbances when in a fatigued or non-fatigued state. Previous experimental evidence has shown that exogenous antioxidant supplementation can inhibit redox signalling pathways and impair the exercise-induced adaptive response. In contrast, the results of Study 3 demonstrated that additional antioxidant support during periods of intensified physical training promoted physiological adaptation and improved performance. These results highlight the need to balance exercise-induced increases in oxidant production and exogenous antioxidant intake to provide a redox environment that is conducive to adaptation. This discovery may also help explain the previously conflicting results regarding the usefulness of antioxidant supplementation in a practical sports setting. Collectively, the findings of this thesis provide novel information regarding the ability to use antioxidant supplementation to manipulate the acute exercise response and training-induced physiological adaptation. However, further research is still required on the topic.

2. Directions for Future Research

To expand upon the findings of this thesis, and develop a greater understanding of the relationship between the oxidative, inflammatory and neuroendocrinological systems in response to exercise-stress, it is recommended that further research investigate,

- Long-term (i.e. 6–12 months) effect of NAC supplementation on physiological adaptation and performance via changes in oxidative and inflammatory markers in well-trained athletes.
- Potential benefit of the periodisation of NAC supplementation within micro- and macro-cycles to match increased training demands to promote physiological adaptation.
- Determine the optimal dosage of NAC for use during both competition and intensified training periods.
- Effect of NAC supplementation on exercise-induced disturbances at a cellular level to establish the effect of increased redox regulated gene expression on

subsequent protein translocation and synthesis on subsequent exercise performance.

- Ascertain if modification in prescribed training loads according to changes in redox markers assists in a better balance between fatigue / recovery and can minimise excessive training-induced fatigue.

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APPENDIX

UNIVERSITY OF TECHNOLOGY, SYDNEY
INFORMED CONSENT FORM

I _____ *(participant's name)* agree to participate in the research project "Nutritional status of elite Australian swimmers: assessing antioxidant intake" being conducted by Katie Slattery at the School of Leisure, Sport and Tourism, Faculty of Business, University of Technology, Sydney.

I understand that the purpose of this study is to determine the nutritional practices of elite swimmers during intensiifed training periods. I understand that my participation in this research may involve up to 12 h of my time over a one week period. I also understand that there are possible risks of participating in this study. These possible risks are:

1. <u>Fatigue from Training:</u> The exercise protocols in the present study will be demanding. It is anticipated that you may feel general fatigue from physical training completed in this study. However, this fatigue will be no greater than you normally endure during training for your sport.

2. <u>Muscle Strains:</u> There is a minor risk of suffering a muscular strain during the exercise being completed during the studies. As the testing in some instances involves maximal force production, it is important for the subject to warm up prior to exercise and warm down at the completion. Leading up to the maximal tests, you will perform activities that gradually build up their muscle temperature to ensure that injury risk is minimised during testing to minimise this risk.

I am aware that I can contact Katie Slattery (phone: 02 9514 5846 or   ) if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason.

I understand that UTS attempts to ensure that the greatest of care will be taken by the researchers during the testing and training sessions. However, I acknowledge that UTS, its agents and employees will not be liable for any loss or damage arising directly or indirectly from these testing and training sessions. I acknowledge and accept that there are risks involved, including but not limited to discomfort, injury and, in extremely rare circumstances, death. I acknowledge and accept that my participation is entirely voluntary, and that UTS has accepted my participation in good faith without express implied warranty.

I agree that Katie Slattery has answered all my questions fully and clearly. I agree that the research data gathered from this project may be published in a form that does not identify me in any way.

_____   ____/____/____
Signed by

_____   ____/____/____
Witnessed by

NOTE: This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer, Ms Susanna Davis (ph: 02 - 9514 1279, Susanna.Davis@uts.edu.au). Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

UNIVERSITY OF TECHNOLOGY, SYDNEY
INFORMED CONSENT FORM

I _____ *(participant's name)* agree to participate in the research project "Time course changes in markers of recovery from competitive team sport matches" being conducted by Katie Slattery and Aaron Coutts at the School of Leisure, Sport and Tourism, Faculty of Business, University of Technology, Sydney. I am aware that my participation in this research may involve up to 18 h of my time over a three week period. I also understand that there are possible risks in participating in this study. These possible risks are:

1. <u>Risk of infection during blood sample collection</u>: There is a very small risk of infection when blood samples are withdrawn during both venipuncture and from the capillarised blood sample taken from a fingertip or earlobe during the studies. However, this risk will minimised. All venipuncture will be performed by a trained phlebotomist in a sterile environment in accordance with the occupational health and safety guidelines outlined by Douglas Hanly Moir Pathology. All capillarised blood sampling will be undertaken by trained personal under sterile conditions using standard procedures.
2. <u>Fatigue from training:</u> The exercise protocols in the present study may be demanding. It is anticipated that you may feel general fatigue from physical training completed in this study. However, this fatigue will be no greater than you normally endure during training for your sport.
3. <u>Muscle strains:</u> There is a minor risk of suffering a muscular strain during the exercise being completed during the studies. As the testing in some instances involves maximal force production, it is important for the subject to warm up prior to exercise and warm down at the completion. Leading up to the maximal tests, you will perform activities that gradually build up their muscle temperature to ensure that injury risk is minimised during testing to minimise this risk.

Throughout the investigation I am aware that pathology testing by Douglas Hanly Moir Pathology will be conducted. In the event of an abnormal result being reported, I understand that my General Practitioner will be contacted by the researchers and an appointment to discuss the results will be arranged. I am aware that Douglas Hanly Moir Pathology are conducting the blood tests in a purely research capacity and are not liable for any medical repercussions arising from the results of the blood tests. I understand that UTS attempts to ensure that the greatest of care will be taken by the researchers during the testing and training sessions. However, I acknowledge that UTS, its agents and employees will not be liable for any loss or damage arising directly or indirectly from these testing and training sessions. I acknowledge and accept that there are risks involved, including but not limited to discomfort, injury and, in extremely rare circumstances, death. I acknowledge and accept that my participation is entirely voluntary, and that UTS has accepted my participation in good faith without express implied warranty. I am aware that I can contact Katie Slattery (phone: 9514 5846 or ■■■■■■) or Aaron Coutts (phone: 9514 5188) if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason. I agree that Katie Slattery and Aaron Coutts have answered all my questions fully and clearly. I agree that the research data gathered from this project may be published in a form that does not identify me in any way.

_____   ____/____/____
Signed by

_____   ____/____/____
Witnessed by

NOTE: This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer, Ms Susanna Davis (ph: 02 - 9514 1279, Susanna.Davis@uts.edu.au). Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

UNIVERSITY OF TECHNOLOGY, SYDNEY

INFORMED CONSENT FORM

I _____ (participant's name) agree to participate in the research project "The effect of acute antioxidant supplementation on endurance performance and adaptation to exercise" being conducted by Katie Slattery at the School of Leisure, Sport and Tourism, Faculty of Business, University of Technology, Sydney in association with the NSW Institute of Sport. I am aware that my participation in this research may involve up to 18 h of my time over a 10 week period. I also understand that there are possible risks in participating in this study. These possible risks are:

1. Risk of infection during blood sample collection: There is a very small risk of infection when blood samples are withdrawn during venipuncture or pinprick. However, this risk will minimised. All venipuncture will be performed by a trained phlebotomist in a sterile environment in accordance with the occupational health and safety guidelines. All capillarised blood sampling from pinprick will be undertaken by trained personal under sterile conditions using standard procedures.

2. Antioxidant Supplementation: The antioxidant N-acetycysteine that will be used in the investigation is a widely used supplement, available without prescription and abides by Australian health and food standards. This antioxidant has been used in previous research and no side effects of supplementation were reported. There is a very minimal risk of adversely reacting to the antioxidant supplement. Prior to participation in the investigation, you will be informed on the contents of both the antioxidant and placebo supplement to ensure you are not allergic to the products.

3. Fatigue from testing: The exercise protocols in the present study may be demanding. It is anticipated that you may feel general fatigue from physical testing completed in this study. However, this fatigue will be no greater than you normally endure during competition.

4. Muscle strains: There is a minor risk of suffering a muscular strain during the exercise completed during the studies. As the testing in some instances involves maximal force production, it is important for the subject to warm up prior to exercise and warm down at the completion. Leading up to the maximal tests, you will perform activities that gradually build up their muscle temperature to ensure that injury risk is minimised during testing to minimise this risk.

I understand that UTS attempts to ensure that the greatest of care will be taken by the researchers during the testing and training sessions. However, I acknowledge that UTS, its agents and employees will not be liable for any loss or damage arising directly or indirectly from these testing and training sessions. I acknowledge and accept that there are risks involved, including but not limited to discomfort, injury and, in extremely rare circumstances, death. I acknowledge and accept that my participation is entirely voluntary, and that UTS has accepted my participation in good faith without express implied warranty.

I am aware that I can contact Katie Slattery (phone: 9763 0204 or [REDACTED]) if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason. I agree that Katie Slattery has answered all my questions fully and clearly. I agree that the research data gathered from this project may be published in a form that does not identify me in any way.

_____   ____/____/____
Signed by

_____   ____/____/____
Witnessed by

NOTE: This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer, Ms Susanna Davis (ph: 02 - 9514 1279, Susanna.Davis@uts.edu.au). Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

08 June 2005

Dr Aaron Coutts
School of Leisure, Sport and Tourism
KG01.06.78
UNIVERSITY OF TECHNOLOGY, SYDNEY

Dear Aaron,

UTS HREC 2005-112 – COUTTS, Dr Aaron (for SLATTERY, Katie May PhD student) – "Time course changes in markers of recovery from competitive team sport matches"

Thank you for your response to my email dated 23/07/05. Your response satisfactorily addresses the concerns and questions raised by the Committee, and I am pleased to inform you that ethics clearance is now granted.

Your clearance number is UTS HREC REF NO. 2005-112

Please note that the ethical conduct of research is an on-going process. The *National Statement on Ethical Conduct in Research Involving Humans* requires us to obtain a report about the progress of the research, and in particular about any changes to the research which may have ethical implications. This report form must be completed at least annually, and at the end of the project (if it takes more than a year). The Ethics Secretariat will contact you when it is time to complete your first report.

I also refer you to the AVCC guidelines relating to the storage of data, which require that data be kept for a minimum of 5 years after publication of research. However, in NSW, longer retention requirements are required for research on human subjects with potential long-term effects, research with long-term environmental effects, or research considered of national or international significance, importance, or controversy. If the data from this research project falls into one of these categories, contact University Records for advice on long-term retention.

If you have any queries about your ethics clearance, or require any amendments to your research in the future, please do not hesitate to contact the Ethics Secretariat at the Research and Innovation Office, on 02 9514 9772.

Yours sincerely,

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Professor Marion Haas
Chairperson
UTS Human Research Ethics Committee

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20 July 2009

Dr Aaron Coutts
School of Leisure, Sport and Tourism
KG01.06.78
UNIVERSITY OF TECHNOLOGY, SYDNEY

Dear Aaron,

UTS HREC 2009-164 – COUTTS, Dr Aaron (for SLATTERY, Katie May PhD student) – "The effect of N-acetylcysteine supplementation on cycling performance and adaptation to exercise - (CLINICAL TRIAL)"

Thank you for your response to my email dated 25/05/09. Your response satisfactorily addresses the concerns and questions raised by the Committee, and I am pleased to inform you that ethics clearance is now granted.

Your clearance number is UTS HREC REF NO. 2009-164A

Please note that the ethical conduct of research is an on-going process. The *National Statement on Ethical Conduct in Research Involving Humans* requires us to obtain a report about the progress of the research, and in particular about any changes to the research which may have ethical implications. This report form must be completed at least annually, and at the end of the project (if it takes more than a year). The Ethics Secretariat will contact you when it is time to complete your first report.

I also refer you to the AVCC guidelines relating to the storage of data, which require that data be kept for a minimum of 5 years after publication of research. However, in NSW, longer retention requirements are required for research on human subjects with potential long-term effects, research with long-term environmental effects, or research considered of national or international significance, importance, or controversy. If the data from this research project falls into one of these categories, contact University Records for advice on long-term retention.

If you have any queries about your ethics clearance, or require any amendments to your research in the future, please do not hesitate to contact the Ethics Secretariat at the Research and Innovation Office, on 02 9514 9772.
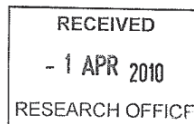
Yours sincerely,

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Professor Marion Haas
Chairperson
UTS Human Research Ethics Committee

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Australian Government

Department of Health and Ageing
Therapeutic Goods Administration

Ref: 2010/000603(133)

Attn: Aaron Coutts
School of Leisure Sport and Tourism
University of Technology – Kuringai Campus
Eton Road
UNDFILED NSW 2070

Dear Mr Coutts,

Re: Clinical Trial Notification for your study – Protocol: N/A
Site: University of Technology – Kuringai Campus

Attached you will find correspondence received by the Therapeutic Goods Administration on **1st March 2010.**

As the requirements under **Item 3 Schedule 5A** of the *Therapeutic Goods Regulations* have not been met, the product cannot be lawfully supplied in the context of a clinical trial. In order for the notification to be processed, please amend the CTN form, as discussed over the phone with Josephine Dufty on the 11 March 2010.

As payment for this trial has not been made, when you have the whole completed form ready, please quote the reference number **2010/000603(133)** and forward it to the following address:

Financial Services Group
Therapeutic Goods Administration
PO Box 100
Woden ACT 2606

If you have any queries please don't hesitate to contact me on (02)

Yours Sincerely,

Production Note:
Signature removed prior to publication.

Katherine Adams
Experimental Drugs Section
Office of Prescription Medicine
Therapeutic Goods Administration
11 March 2010