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

A thesis submitted for the degree

Doctor of Philosophy

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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

Acknowledgements

'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.
- **Slattery, K.M.**, Wallace, L.K., Coutts, A.J., & Bentley, D.J. (2007). *Effects of High vs. Low training loads on metabolic, immune and oxidative markers in team sport athletes*. Paper presented at the Australian Conference of Science and Medicine in Sport, Adelaide, Australia.

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%	75%		30%	10%	60%		10%	10%	20%	60%

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_2O_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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