



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

A thesis submitted for the degree

Doctor of Philosophy

March 2012

By

Katie May Slattery

Bachelor of Arts in Sports and Exercise Management

Bachelor of Arts (Honours) in Human Movement

Sport & Exercise Discipline Group

UTS: Health

University of Technology

Sydney, Australia

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.

Katie Slattery

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.

Associate Professor Aaron Coutts truly deserves the MVP award. Time is one of the most precious gifts you have, and you are always willing to give yours ... for the full 7 years it has taken me to finish this thesis. Thank you for your superb guidance and mentorship.

Honourable mention goes to Lee Wallace, who instilled my enthusiasm for sports science. Thank you for your encouragement, insight and feedback throughout my doctoral studies.

I would also like to thank Dr David Bentley my co-supervisor for his assistance and valuable advice.

Thank you to Dr Greg Peoples, Adam Zieba, Dr Ben Dascombe, Dr Dean Scully, Erin McCleave and Anna Ross for their assistance with data collection and analysis. The participants and coaches who readily volunteered to be part of the investigations. Moreover, thank you to editor extraordinaire, David Young.

I am grateful to the NSW Institute of Sport for their support and flexibility in allowing me to balance work commitments and study. Particularly, my manager, Robert Medicott and Kenneth Graham in the Applied Research Department.

I would like to acknowledge the University of New South Wales, the University of Wollongong and the University of Newcastle for allowing access to facilities and equipment.

This research was supported by a UTS Faculty Grant, Australian Sport Commission General Collaborative funding, Douglas Hanly Moir Pathology and the NSW Institute of Sport.

Finally, to my family and friends, a huge thank you for all of your encouragement, well-wishes, patience and unwavering support.

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 adaptation 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
<i>d</i>	Cohen's <i>d</i> effect size
DAG	diacylglycerol
DALDA	Daily Analysis of Life Demands for Athletes
F₂-isoprostane	15-isoprostane F _{2t} concentration
FRAP	ferric reducing ability of plasma
FT₃	free triiodothyronine
FT₄	free thyroxine
<i>g</i>	Hedge's <i>g</i> effect size
GH	growth hormone
Gr	glutathione reductase
GPX	glutathione peroxidase
GSH	reduced glutathione
GSH:GSSG	reduced glutathione to glutathione ratio
GSSG	glutathione
HIGH	intensified training period
H₂O₂	hydrogen peroxide;
HSF	heat shock factor
HSP	heat shock protein
ICC	intra-class correlation
IκB	inhibitor -kappaB
IκK	inhibitor - kappaB kinase
IL-6	interleukin-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
η²_p	partial eta squared
NAC	N-acetylcysteine

NADPH	nicotinamide-adenine dinucleotide phosphate
NF-κB	nuclear factor-kappaB
nm	nanometre
NMT	non-motorised treadmill
O₂	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

Table of Contents

Certificate of Authorship and Originality of Thesis	i
Preface.....	iii
List of Articles Submitted for Publication.....	iv
Refereed Journal Publications.....	iv
Conference Proceedings & Abstracts.....	iv
Statement of Candidate Contribution	i
Abstract	i
Keywords	ii
List of Abbreviations.....	iii
Table of Contents	v
List of Figures.....	viii
List of Tables	x
CHAPTER ONE	1
Introduction.....	1
1. Background.....	2
2. Research Problem.....	4
3. Study Objectives	4
CHAPTER TWO	7
Literature Review	7
Abstract.....	8
The Stress-Response to Exercise.....	9
Free Radical Biochemistry and Exercise.....	10
Affect of Antioxidant Supplementation on Performance	20
Interplay between Inflammatory, Hormonal and Oxidative Response during Exercise	25
Limitations and Directions for Future Research	33
Summary and Conclusions	33
CHAPTER THREE	35
Antioxidant intake of well-trained athletes during intensified physical training.....	35
Abstract.....	36
Introduction	37
Methods	38
Results.....	39
Discussion.....	41

CHAPTER FOUR	44
Effect of training load on simulated team sport match performance	44
Abstract	45
Introduction	46
Methods	47
Results	52
Discussion.....	57
CHAPTER FIVE.....	61
Evaluating the effect of acute changes in training load on select endocrinological and oxidative damage markers in team sport players	61
Abstract	62
Introduction	63
Methods	65
Results	69
Discussion.....	72
CHAPTER SIX.....	76
The effect of N-acetylcysteine on cycling performance following intensified training in well-trained triathletes: a double blind randomised placebo controlled study	76
Abstract	77
Introduction	78
Methods	81
Results	87
Discussion.....	94
CHAPTER SEVEN	98
General Discussion	98
1. Main Findings	99
2. Limitations	102
3. Practical Applications	103
CHAPTER EIGHT.....	104
Summary and Recommendations	104
1. Thesis Summary.....	105
2. Directions for Future Research.....	107

REFERENCES	109
APPENDIX	124

List of Figures

Figure 2.1: A schematic diagram illustrating the redox signalling pathways for skeletal muscle adaptation.	12
Figure 2.2: The effect size of antioxidants to improve exercise performance.	22
Figure 2.3: The hormetic effect of exercise parameters on performance.	32
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.	41
Figure 3.2: Mean 4 d training load and corresponding 'worse than normal' DALDA score for each participant.	41
Figure 4.1: Experimental design.	48
Figure 4.2: Training program for the LOW (a) and HIGH (b) experimental periods.	50
Figure 4.3: 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.	53
Figure 4.4: Peak sprint velocities reached during sprint throughout the HIGH and LOW match.	54
Figure 4.5: Capillary blood lactate concentration during the match simulation following the HIGH and LOW training loads.	54
Figure 4.6: 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.	56
Figure 4.7: Pre- to post-match simulation changes in plasma monocyte chemoattractant protein-1 (MCP-1) following the HIGH and LOW training loads.	57
Figure 5.1: Training program for the LOW (a) and HIGH (b) experimental periods.	67
Figure 5.2: F ₂ -isoprostane during the high-intensity intermittent running protocol following the HIGH and LOW training conditions.	70
Figure 5.3: 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.	71
Figure 6.1: Schematic diagram of the experimental design. Cycle ergometer race simulation, Venous blood and urinary samples.	83
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.	88

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 \pm SD)..... 90

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)..... 92

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)..... 92

Figure 6.6: Time course of changes in A. plasma interleukin-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)..... 93

Figure 6.7: Time course of changes in peripheral mononuclear cell extract nuclear factor-kappaB (NF- κ B). 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)..... 94

Figure 7.1: Effect of exogenous antioxidant supplementation dependent on an athlete's level of fatigue. 102

List of Tables

Table A: Percentage contribution (%) of each author to the investigations conducted during the candidature.....	i
Table 2.1: Summary of previous investigations on oxidative stress measures following intensified training.	15
Table 3.1: Mean nutritional intake for well-trained athletes during a 4 d intensive training period.	40
Table 6.1: Cycle ergometer race simulation protocol.	84
Table 6.2: Time trial performance parameters before and after supplementation with both NAC and placebo.....	89
Table 8.1: Summary of the investigations conducted as part of the thesis.	106