Vitamin C and Exercise-Induced Oxidative and Inflammatory Stress in Ultramarathon Athletes

by

Edith Margret Futre (nee Peters)
BA Hons (University of Stellenbosch),
BSc (Med) Hons and M Sc (Med) (University of Cape Town)

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Department of Immunology, Faculty of Medicine, University of Pretoria

Pretoria

April 2002

Abstract

Literature reveals a paradoxical response of the immune and innate host defence systems to endurance exercise; apparent stimulation following long-term regular training and suppression in response to acute exposure to exhaustive endurance exercise. Two epidemiological studies revealed that clinical manifestation of immunosuppression in the form of increased incidence of upper respiratory tract infection symptoms (URTI) is greater following competitive ultramarathon running than in matched sedentary controls and linearly related to running time. These were followed by three intervention studies in which the efficacy of anti-oxidant supplementation in reducing the incidence of post-race symptoms of infection was assessed following participation in the 90 km Comrades Ultramarathon. Vitamin C alone was found to be more effective than combinations of anti-oxidants in reducing the post-race incidence of URTI symptoms in a study conducted on 178 runners and 162 matched, sedentary controls.

In order to investigate mechanisms by which vitamin C may act in reducing the incidence of URTI infection during the two-week post-race period, intervention studies were conducted at the 1997 and 1999 90 km Comrades Ultramarathons. Runners were required to keep dietary records and ingest prescribed capsules containing 500 mg, 1000 mg, 1500 mg vitamin C or placebo for 10 days prior to the event, complete the 90 km ultramarathon and provide 35 ml blood 14-16 hours before, immediately on completion of, 24 hours and 48 hours post-race. These specimens were subsequently assayed for markers of inflammatory and oxidative stress which included circulating cortisol, adrenaline, vitamin A, vitamin C, vitamin E, C-reactive protein (CRP), serum amyloid A, creatine kinase, lactate dehydrogenase, neutrophils and lymphocytes, neutrophil-derived elastase and myeloperoxidase, the pro-inflammatory-cytokines, interleukin-1ß, interleukin-6, interleukin-8 and tumour necrosis factor-alpha, the anti-inflammatory-cytokine, interleukin-10 (IL-10), and IL-1 receptor antagonist (IL-1Ra), all of which (with the

exception of vitamins A and E and lymphocytes) were significantly elevated on completion of the ultramarathon.

Increased daily intake of vitamin C was accompanied by a dose-related attenuation of the exercise-induced increases in circulating vitamin C, cortisol and neutrophils. Supplementation with the vitamin at doses of ≤ 1000 mg daily appeared to result in augmentation of the exercise-induced inflammatory response as evidenced by significant increases ($p<0.05$) in circulating levels of the acute phase reactant, CRP and in the group receiving 1000 mg daily, an increase in creatine kinase. In the group receiving 1500 mg daily, this apparent pro-inflammatory effect of supplementation with the vitamin was less evident, but circulating cortisol, adrenaline, IL-10 and IL-1Ra concentrations were all significantly reduced ($p<0.05$), suggesting that at this level of supplementation the vitamin may activate a counteracting anti-inflammatory cascade, possibly through inhibition of activation of pro-inflammatory transcription factors.

Although further work involving larger sample sizes is required to confirm these findings, this is the first evidence that vitamin C supplementation attenuates the endogenous, biological anti-inflammatory response to intensive exercise, which may partially explain why the vitamin reduces the incidence of URTI in ultramarathon athletes. On the downside, however, apparent augmentation of exercise-associated inflammatory responses at supplementation levels of 500 and 1000 mg daily, is clearly of concern.

Key Words: **Upper respiratory tract infections, ultramarathon running, vitamin C, oxidative stress, inflammatory stress, cortisol, adrenaline, interleukin-10, interleukin-1Ra, C-reactive protein, amyloid A.**

Abstrak

Die literatuur toon 'n teenstrydige reaksie van die immuun en intrinsieke gasheer weestandsmeganismes ten opsigte van uithouvermoë-oefening; die klaarblyklike stimulasie daarvan as gevolg van langtermyn, gereelde oefening maar ook die demping daarvan wat volg na akute blootstelling aan uitputtende uithouvermoë-oefeninge. Twee epidemiologiese studies het aangedui dat die kliniese manifestasie van immuunonderdrukking in die vorm van 'n verhoging in voorkoms van boonste lugweg infeksie-simptome groter was na mededingende ultramaraton wedlope as in rustende kontroles. Verder, dat hierdie simptome direk verwant was aan die hardlooptyd. Hierdie studie is opgevolg deur drie ander farmakologiese ingrypingstudies waarin die doeltreffendheid van anti-oksidant aanvullings om 'n vermindering in voorkoms van na-wedloopsimptome teweeg te bring, ondersoek is in deelnemers van die 90 km Comrades Ultramaraton. In 'n studiegroep bestaande uit 178 deelnemers en 162 rustende kontroles is gevind dat vitamien C alleen meer effektief was as kombinasies van anti-oksidante, om die na-wedloop voorkoms van boonste lugweg infeksie-simptome te verminder.

Ten einde die meganismes vas te stel waardeur vitamien C die voorkoms van boonste lugweg-infeksie gedurende die twee week- periode na die wedloop verminder, is twee ingrypingstudies onderneem tydens die 1997 en 1999 90 km Comrades Ultramaratons. Deelnemers is versoek om boek te hou van hul dieet, voorgeskrewe kapsules van 500 mg, 1000 mg, 1500 mg vitamien C of plasebo te neem, die 90 km ultramaraton te voltooi en om 35 ml bloed 14-16 uur vooraf, onmiddellik by voltooiing van, 24 uur en 48 uur na die wedloop te skenk. Die bloedmonsters is daarna getoets vir merkers van inflammatoriese en oksidatiewe stres wat insluit: sirkulerende kortisol, adrenalien, vitamien A, vitamien C, vitamien E, C-reaktiewe proteïen (CRP), serum amiloïed A (SAA), kreatien kinase, laktaat dehidrogenase, neutrofiel-afkomstige elastase en miëloperoksidase (MPO), die pro-inflammatoriese sitokiene, IL-1β, IL-6, IL-8, TNF-α, die anti-inflammatoriese sitokien, IL-10 en IL-1 reseptor antagonis (IL-1Ra).

inflammatoriese sitokiene, IL-1β, IL-6, IL-8, TNF-α, die anti-inflammatoriese sitokien, IL-10 en IL-1 reseptor antagonis (IL-1Ra).

Die verhoogde daaglikse inname van vitamien C het gepaard gegaan met 'n dosis-verwante vermindering van oefeningsgeïnduseerde verhogings in sirkulerende vitamien C, kortisol en neutrofiele, sowel as 'n daling in die limfosiete ($p<0.05$). Supplementasie met die vitamien by doserings van ≤1000 mg daagliks het klaarblyklik tot 'n verhoging in die oefeningsgeïnduseerde inflammatoriese respons gelei, soos aangedui deur betekenisvolle verhogings ($p<0.05$) in die sirkulerende vlakke van die akute fase reagens, CRP, en in die groep wat 1000 mg daagliks ontvang het, 'n verhoging in kreatien kinase. In die groep wat daagliks 1500 mg ontvang het, was die klaarblyklike pro-inflammatoriese uitwerking van die vitamienaanvulling minder opsigtelik, maar was konsentrasies van sirkulerende kortisol, adrenalien, IL-10, en IL-1Ra almal betekenisvol minder ($p<0.05$) wat aandui dat by hierdie vlak van supplementasie, die vitamien 'n teenwerkende anti-inflammatoriese kaskade, waarskynlik deur die demping van aktivering van pro-inflammatoriese transkripsie-faktore, aktiveer.

Hoewel verdere studies groter studiegroepe vereis om hierdie bevindings te bevestig, is dit die eerste bewys dat vitamien C aanvullings die endogene, biologiese anti-inflammatoriese respons ten opsigte van intensiewe oefening demp, wat mag verklaar waarom die vitamien die voorkoms van boonste lugweginfeksie in ultra-maraton atlete verminder. In teendeel is die klaarblyklike verhoging van oefeningsverwante inflammatoriese response by aanvullingsvlakke van 500 en 1000 mg daagliks, kommerwekkend.

Kernwoorde: **Boonste lugweg infeksies, ultramaraton wedlope, vitamien C, oksidatiewe en inflammatoriese stres, kortisol, adrenalien, interleukin-10, interleukin-1Ra, C-reaktiewe proteïen, amiloïed A.**

Acknowledgements

I would like to express my sincere gratitude to all who have assisted me in the work which has resulted in the final submission of this thesis. A particular word of thanks is extended to the following persons :

- My supervisor, **Professor R Anderson**, Head of the Department of Immunology, Medical Faculty, Pretoria University, for sharing with me his insight of and specific interest in Vitamin C and oxidative stress, and for all the hours of patient assistance over the last five years. It was indeed a privilege to have been able to work under your guidance.

- **Professor Tim Noakes,** Director of the South African Institute of Sport Science, for introducing me to the broader field of Sport Science and for being a constant source of support and inspiration to me throughout my asssociation with this field of study.

- **Dr David Nieman**, Head of the Department of Health and Exercise Science, Appalachian State University, USA, for the personal support and encouragement to complete this work. I should also like to acknowledge the contribution of his laboratory with the analysis of the cytokine concentrations in the collaborative work of the 1999 study (reported in Chapter Six).

- **Prof Eric Bateman**, Head of the Department of Medicine, Cape Town University, for instigating the original epidemiological study in 1982, which was to introduce me to the field of exercise immunology.

- **Prof Annette Theron,** Associate Professor in the Department of Immunology, University of Pretoria, for expert laboratory assistance, and also the friendship and support over the last five years.

- **Mrs Heidi Fickl**, biomedical scientist in the Department of Immunology, University of Pretoria, for outstanding laboratory assistance, for her thoroughness in teaching me laboratory skills, in particular the development of appropriate ELISA techniques for use in this work and for letting me work in her laboratory. I was indeed privileged to have obtained such training.

- **Mrs Cathy Connolly** from the Biostatistics Unit of the Medical Research Council, Natal for valuable assistance with statistical computations.

- **Dr Vinod Jogessar and Dr Vincent Naiker** from the Department of Haematology of the Nelson Mandela Medical School, Natal University, for providing assistance with the analysis of full blood counts and differential lymphocyte counts in the 1999 study.

- **Dr Elena Nevines,** for always being available to share her expert knowledge of chemistry, her assistance with collation of the original data, patience in teaching me the skills of compiling graphic material, and general moral support during my years in the Department of Physiology.

- **Jeni Goetzsche** and **Lester Joseph** for their assistance with the intervention study conducted at the 1993 Comrades Marathon, **Tim Lucas** and **Desiree Lee** for assistance with the field studies conducted at the 1996 and 1997 Comrades Marathons and to **Andrew McKune** and my research team from the Department of Physiology at the 1999 Comrades Marathon.

- **UNESCO,** the **University of Natal and University of the Witwatersrand Research Funds**, and **Institute of Pathology, University of Pretoria Medical School** for financial contributions to this research. The assistance of the **Roche Pty Ltd** and **Pharma Natura Pty (Ltd)** in formulating and providing the placebo and anti-oxidant supplements used in these studies is also acknowledged.

- **the more than 300 runners** who volunteered to participate in the various studies which I have conducted at the 1993, 1996, 1997 and 1999 Comrades Marathon events. Without your unselfish co-operation, the data reported in this thesis would not have been available.

- **Professor F Güldner,** Head of the School of Medical Sciences, **and my colleagues** in the Department of Physiology of the Nelson Mandela Medical School, Natal University, for granting me the sabbatical leave which provided relief from my teaching responsibilities and the opportunity to attend to the write-up of this thesis. Without this, it would been physically impossible to complete this work.

- **And finally, to my daughter, Fia,** thank you for your encouragement and support and your tolerance of my constant occupation of our home study; **and to my extended family and friends**, thank you, also, for your support and for tolerating my preoccupation with this work over the last few years.

Table of Contents

List of Figures

List of Tables

Table 7.3: Mean (±SEM) plasma interleukin-8 and elastase concentrations.

Recent Publications
which have emanated from this work

Pilot Work (Field Study)

- **Peters E.M.**, Goetzsche J.M., Joseph, L.E., Noakes T.D. Vitamin C as effective as combinations of anti-oxidant nutrients in reducing the incidence of upper respiratory tract infections in ultradistance runners. *S.A. Sports Med.* **4**: 23-27, 1996.

Laboratory Work

- **Peters E.M.**, Anderson, R., Theron, A.J. Attenuation of the increase in circulating cortisol and enhancement of the acute phase response in vitamin C-supplemented ultramarathon runners. *Int J Sports Med* **22**: 120-126, 2001.
- Nieman D.C., **Peters E.M.**, Henson D.A., Nevines E.I., Thompson M.M. Influence of Vitamin C supplementation on cytokine changes following an ultramarathon. *J Interferon & Cytokine Res* **20**: 1029-35, 2000.
- **Peters E.M.**, Anderson, R., Nieman D.C., Fickl H., Joggesar V. Vitamin C supplementation attenuates the increases in circulating cortisol, adrenaline and anti-inflammatory polypeptides following ultramarathon running. *Int J Sports Med* ***22***:537-543, 2001.

Review Articles

- **Peters E.M.** Exercise and Upper Respiratory Tract Infections. *S Afr J Sports Med* **3:** 9-14, 1996.
- **Peters E.M.** Exercise, immunology and upper respiratory tract infections, *Int J Sports Med* **18**, S69-78, 1997.
- **Peters-Futre E.M.** Exercise, Vitamin C and Neutrophil Function. The Missing Link. *Exerc Immunol Rev* **3**: 32-52, 1997.

Chapter in Book

- **Peters E.M.** Vitamins, Immunity and Infection Risk in Athletes In: Nieman D.C., Pederson BK (eds): Nutrition and exercise Immunology, CRC Press, Florida, 2000.

Abbreviations

8-oxodG	8-oxo-2-deoxyguanosine
AA	ascorbic acid
ACTH	adrenocorticotrophic hormone
ANOVA	analysis of variance
AP-1	activator protein-1
APP	acute phase proteins
AVP	arginine vasopressin
CK	creatine kinase
CRF	corticotropin-releasing hormone
CRP	C-reactive protein
CSIF	cytokine synthesis inhibiting factor
Cu	Copper
DHAA	dehydro-ascorbic acid (oxidised form)
DOMS	delayed onset muscle soreness
EBV	Epstein-Barr Virus
EDTA	ethylenediaminetetraacetic acid
FMLP	N-formyl-methionyl-leucyl-phenylalanine
H_2O_2	hydrogen peroxide
HPA	hypothalamic-pituitary-adrenal
HOCL	hypochlorous acid
HPLC	high performance liquid chromatography
IE	infectious episodes
IgA	immunoglobulin A
IL-1β	interleukin-1 beta
IL-1Ra	interleukin-1 receptor antagonist
IL-2	interleukin-2
IL-2R	interleukin-2 receptor
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10

IKK	I-kappa B kinase
L-ascorbate	reduced form of ascorbic acid
LDL	low density lipoprotein
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
MIP	macrophage inhibitory protein
MPO	myeloperoxidase
MSH	alpha-melanocyte stimulating hormone
NF*k*B	nuclear transcription factor, *k*B
NK cells	natural killer cells
OH^-	hydroxyl anion
•OH	hydroxyl radical
$O_2^{\bullet -}$	superoxide anion
PBS	phosphate-buffered saline
PMA	phorbol 12-myristate 13-acetate
PMN	polymorphonuclear leukocyte
PWM	pokeweed mitogen
ROI	reactive oxygen intermediates
ROS	reactive oxygen species
SAA	serum amyloid A
SD	standard deviation
SEM	standard error of the mean
SMI	steroid-mediated immunosuppression
SOD	superoxide dismutase
TNFα	tumour necrosis factor alpha
URT	Upper respiratory tract
URTI	Upper respiratory tract infections
VC	Vitamin C
$\dot{V}O_2$ max	Maximum oxygen consumption