ORIGINAL RESEARCH ARTICLE

Alterations in acute-phase reactants (CRP, rheumatoid factor, complement, Factor B, and immune complexes) following an ultramarathon

S J Semple (MTech)[1]
L L Smith (PhD)[1]
A J McKune (MMedSci)[1]
N Neveling (BA Hons)[1]
A Wadee (PhD)[2]

[1]Department of Sport and Physical Rehabilitation Sciences, Tshwane University of Technology, Pretoria
[2]Department of Immunology, University of the Witwatersrand Johannesburg

Abstract

Objectives. The human body initiates an acute phase response (APR) in response to a wide range of homeostatic disturbances. This complex series of reactions serves to activate repair processes and prevent ongoing tissue damage. An important aspect of the APR is the *de novo* synthesis of acute phase proteins (APP), many of which have not been thoroughly investigated.

Main outcome measures. Alterations in CRP (C-reactive protein), C1est, C3, C4, C6, rheumatoid factor (RF) and Factor B were determined before and after an ultramarathon. Data were analysed using a one-way analysis of variance comparing values to pre-exercise levels. Significance was set at $p < 0.05$.

Design. Venepunctures were performed on athletes participating in an ultramarathon (90 km) 24 hours before, immediately post-exercise (IPE), and 3h, 24h and 72h after the race. Serum was stored at –80°C until analysed. CRP levels in serum were assessed using the N Latex CRP kit. The levels of circulating immune complexes (CIC) were determined using particle-enhanced nephelometry. Complement proteins C1est, C3, C4 and RF were measured using laser nephelometry. C6 and Factor B were determined by radial immunodiffusion.

Results. CRP was significantly elevated IPE (58%), 3h post (77%), 24h post (87%) and 72h post (69%). Pre-race CRP levels were above the normative range (5.10 ± 3.08 mg/l), C6 was significantly elevated ($p < 0.05$) at 24h post (7.8%) and 72h post (8.8%) exercise. Factor B was significantly elevated ($p < 0.05$) at 72h post exercise (12.8%). RF was significantly elevated at 72h post exercise (6.7%).

Conclusion. Significant increases in selected acute-phase reactants occur several days after the exercise event. In addition, as indicated by elevated resting levels of CRP, the athletes began the race with some degree of inflammation, presumably as a result of the cumulative training and racing mileage in preparation for the ultramarathon.

CORRESPONDENCE:

S J Semple
Private Bag X680
Pretoria
0001
Tel: 012-318 4324
Fax: 012-318 5801
E-mail: semplesj@tut.ac.za

Introduction

In response to a wide range of homeostatic disturbances, such as trauma, neoplasms, bacterial infection, burn injury and immunologically mediated inflammatory states,[7,10] the body initiates an acute phase response (APR). The APR is synonymous with alterations in circulating acute phase proteins (APP), the production of which is mainly due to *de novo* synthesis.[20] This complex series of reactions serves to activate repair processes and prevents ongoing tissue damage by altering metabolic, endocrinological, neurological and immunological functions.[6] Strenuous exercise has been shown to elicit an increase in APP.[3,11,21,30]

Documentation of the acute phase reactants response to physical activity is important when interpreting metabolic/biochemical adaptations and/or maladaptations that occur with exercise.[11] In addition, it provides crucial information with regard to inflammation, healing and adaptation to training stimuli.

Whilst numerous studies have shown the APP C-reactive protein (CRP) to be significantly elevated following exercise,[18,21,26,27] there still remain a large number of acute phase reactants that have not been thoroughly investigated.

Therefore, the aims of this study were firstly to confirm the reported increases in CRP following a prolonged, strenuous bout of exercise, and secondly to monitor alterations in less researched acute phase reactants, up to 3 days after an ultramarathon.

Methods

Subjects

The study was approved by the institutional Ethics Committee and all subjects provided written, informed consent. Six male and 5 female experienced ultramarathon athletes volunteered for the study. The subjects descriptive characteristics were (mean (± SD)): age 43 ± 9 years, height 170 ± 10 cm, weight 64 ± 13 kg, body fat 14 ± 2.9%, maximal oxygen intake (VO_{2max}) 57.5 ± 5.5 ml/kg/min, mileage from January to June 1 450 ± 445 km, and number of completed ultramarathons (90 km) 4 ± 1.

Blood samples

Venous blood was drawn from the subjects 24h before their predicted finishing time, immediately post exercise (IPE), 3h post exercise and then at 24h and 72h after an ultramarathon (90 km). Concentrations were corrected for changes in plasma volume IPE, but not for the remaining time measurements. Corrections for changes in plasma volume were calculated using haematocrit and haemoglobin values in accordance with the methods of Dill and Costill.[5] Samples were allowed to stand for 15 minutes, after which the serum was centrifuged at 1 000 g for 10 minutes. Samples were stored at –80ºC until analysed. All samples were analysed in duplicate.

CRP and rheumatoid factor

Levels of CRP and rheumatoid factor (RF) in serum were determined using the N Latex CRP kit (Behring Diagnostics, Germany). Specimens were mixed with polystyrene particles coated with monoclonal antibodies and the intensity of light scatter measured in a Behring nephelometer (Behring Diagnostics, Germany) against standards of known concentration.

Circulating immune complexes

The levels of circulating immune complexes (CIC) levels were determined using particle-enhanced nephelometry. The assay utilised polystyrene particles coated with human C1q which was added to the subjects' sera. Light scatter due to agglutination of the C1q-coated particles in the presence of CIC was measured in a Behring nephelometer (Behring Diagnostics, Germany) whereby the concentration of CIC was determined in relation to the amount of agglutination detected.

C1est, C3 and C4

Determination of complement proteins C1est, C3 and C4 in serum was performed using specific anti-sera to C1est, C3c and C4. The immune complexes formed were measured in a Behring nephelometer (Behring Diagnostics, Germany) and the amount of C1est, C3 and C4 was calculated by comparison with standards of known concentration.

C6 and Factor B

C6 and Factor B were determined by radial immunodiffusion (The Binding Site, UK). The assays were performed by adding serum and controls of known C6 and Factor B concentrations to radial immunodiffusion plates containing non-specific antibody in an agarose gel. The diameter of immunoprecipitating antigen-antibody complexes radiating out of the wells was measured and compared against a calibrated curve drawn from a range of samples of known concentration.

Statistical analysis

Data were analysed using a one-way analysis of variance (ANOVA), contrasting variables to baseline values. Significance was set at $p \leq 0.05$. Where appropriate *post hoc* tests (Tukey) were computed. SAS statistical software package was used to compute results.

Results

CRP was significantly elevated ($p = 0.003$) by 58% immediately after the race and was significantly increased ($p = 0.001$) by 77% 3h after the race. CRP remained significantly elevated ($p = 0.011$) and peaked at 24h post marathon (87%). At 72h post marathon, CRP was still significantly elevated ($p = 0.0002$), but began returning to baseline (69%) (Fig. 1). The 24h pre-race sample of 5.10 (± 3.08 mg/l) was elevated above normative ranges for the laboratory in which the assays were performed (Table I).

C6 was significantly elevated ($p = 0.03$) at 24h post marathon (7.8%) and remained significantly elevated ($p = 0.02$) at 72h post exercise (8.8%) (Fig. 2).

Factor B was significantly elevated ($p = 0.04$) at 72h post exercise (12.8%) (Fig. 3). Although significantly elevated at 72h post exercise (296.56 ± 35.61 mg/l), this still remained within normative ranges (Table I).

RF was significantly elevated ($p = 0.03$) at 72h post exer-

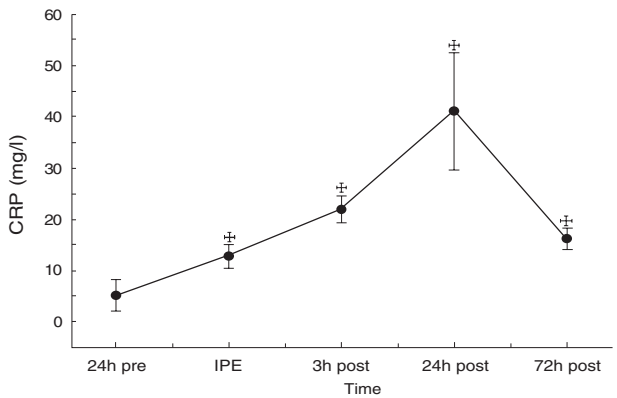

Fig. 1. Alterations in C-reactive protein (mg/dl) of 6 male and 5 female subjects following completion of an ultramarathon. (‡ Signifies p < 0.05 compared with baseline levels.)

TABLE I. Normative ranges	
Variable	**Normative ranges**
C-reactive protein (mg/l)	< 5
C3 (g/l)	0.5 - 1.53
C4 (g/l)	0.2 - 1.00
C6 (mg/l)	45 - 96
Factor B (mg/l)	191 - 382
C1-esterase inhibitor (mg/dl)	31 - 43
Circulating immune complexes (µg/ml)	< 5
Rheumatoid factor (IU/ml)	< 11

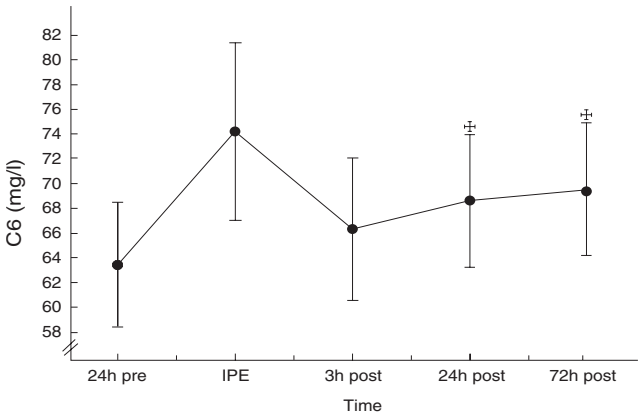

Fig. 2. Alterations in C6 (mg/l) of 6 male and 5 female subjects following completion of an ultramarathon. (‡ Signifies p < 0.05 compared with baseline levels.)

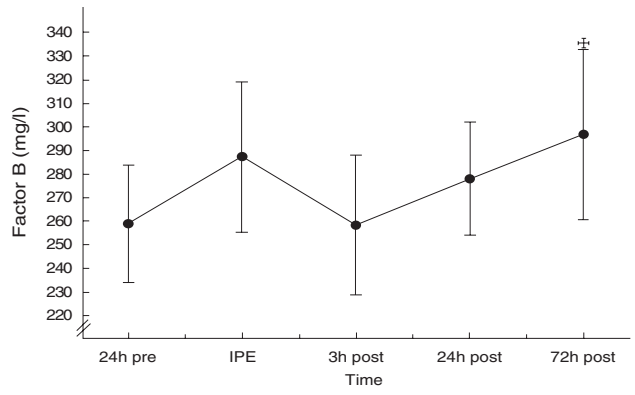

Fig. 3. Alterations in Factor B (mg/l) of 6 male and 5 female subjects following completion of an ultra-marathon. (‡ Signifies p < 0.05 compared with baseline levels).

cise (6.7%) (Fig. 4). As with CRP, RF at baseline (12.12 IU/ml) was elevated above normative ranges (Table I).

C4, C3, CIC and C1est showed no significant alterations up to and including 72h post exercise, and remained consistently within the normative ranges (Table I).

Plasma volumes changed by –2.66 (±4.04%) immediately post exercise.

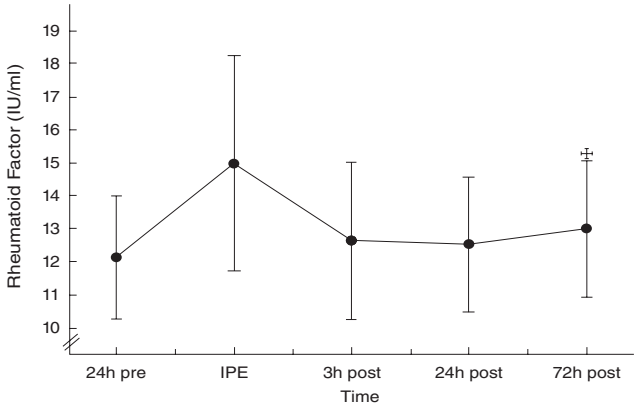

Fig. 4. Alterations in rheumatoid factor (IU/ml) of 6 male and 5 female subjects following completion of an ultramarathon. (‡ Signifies p < 0.05 compared with baseline levels.)

Discussion

This study supports previous research in reporting a significantly increased level of the positive APP, CRP following a strenuous prolonged bout of exercise. CRP, a member of the pentraxin family of proteins has traditionally been used in a clinical setting as an indicator of inflammation.[7] CRP reacts with cell surface receptors to facilitate opsonisation, enhances phagocytosis, acts as a potent stimulator of the complement pathway and modulates polymorphonuclear (PMN) function.[10,20,25] CRP in the present study was significantly elevated IPE and peaked 24h after the race (Fig. 1), a finding mirroring that of Strachan *et al.* [26] and Peters *et al*.[21] Whilst prolonged strenuous exercise such as marathon running seems to elicit a reaction analogous to an APR, research by Fallon *et al*.[12] has shown that the training typical of elite female netball and soccer teams is not associated with significant changes in CRP. Following 9 months of training, the resting levels of CRP in the netball and soccer players was 1.66 mg/l (± 0.89) and 1.62 mg/l (± 1.32) respectively.

Conversely Meyer *et al*.[18] showed a significant increase in CRP 24h following an anaerobic cycling session. It was suggested that athletes/physicians should interpret these elevated levels of CRP as a sign that the inflammatory response has not resolved and thus the athlete should reduce or limit the number of sessions that induce this response.

CRP at baseline (24h premarathon) was slightly elevated above the normal range (Table II). These are norms established for the general South African population and not specifically for sportsmen/women.

Due to the nature of the sport, the elevated CRP levels at rest (5.10 ± 3.08 mg/l) could be attributed to cumulative exercise-induced muscle damage. The average individual has a CRP concentration of 2 mg/l and whilst concentrations greater than 5 mg/l have been associated with acute infections, anything less than 10 mg/l has been regarded as clinically unimportant.[10] This raises the question of whether athletes have elevated resting levels of CRP as a result of

TABLE II. Acute phase reactants showing no significant changes post exercise (mean ± SE)

	24h pre	IPE	3h post	2h post	72h post
C4 (g/l)	0.27	0.24	0.23	0.20	0.21
	(± 0.03)	(± 0.04)	(± 0.04)	(± 0.01)	(± 0.01)
C3 (g/l)	1.10	1.15	1.04	1.00	1.02
	(± 0.12)	(± 0.11)	(± 0.07)	(± 0.05)	(± 0.05)
CIC (ug/ml)	1.57	1.43	1.51	1.32	1.29
	(± 0.15)	(± 0.16)	(± 1.36)	(± 0.12)	(± 0.10)
C1est (mg/dl)	23.51	25.53	23.32	23.78	24.62
	(± 0.75)	(± 1.87)	(± 0.61)	(± 0.92)	(± 0.77)

CIC = circulating immune complexes; C1est = C1-esterase; IPE = immediately post exercise.

continuous chronic exercise. If so, does the increased CRP serve to provide the host with an 'enhanced' immune function, or does it merely represent an adaptation to the continuous inflammation experienced as a result of mechanical stress? CRP exhibits both pro and anti-inflammatory actions. Whilst it is involved in activating complement and opsonising bacteria/pathogens/debris[7] (pro-inflammatory), its net effect is anti-inflammatory in that it has been shown to prevent adhesion of neutrophils to endothelial cells, to inhibit the generation of superoxide by neutrophils and to stimulate the synthesis of interleukin-1 receptor antagonist.[10] Thus, it is arguable that the increased CRP may serve to limit the negative effects associated with repeated bouts of exercise.

Although elevated resting levels of CRP were shown in this study and have been shown elsewhere,[14,27] more studies seem to be revealing that athletes exhibit a chronic training/exercise-induced reduction in resting CRP concentrations.[17,22,29] Whether these suppressed resting levels of CRP increase an individual's susceptibility to infection remains to be investigated.

Prolonged and/or strenuous exercise is associated with muscle damage/trauma. [4,13] This disruption of homeostasis activates complement, a pathway that is central to the development of inflammation. Complement serves to control inflammatory reactions and chemotaxis, assists with the clearance of immune complexes, activates cells and elicits antimicrobial defences.[1, 23]

C6, a less-researched terminal complement component, forms part of the membrane attack complex (MAC) and was significantly elevated at 24h and 72h after the race (Fig. 2). Although C5 was not measured in the present study, previous research has shown C5a (the active inflammatory mediator of C5) to be significantly elevated following exercise.[2] This would imply an increase in C5, which forms the beginning of the MAC. Elevation of C6 in the present study, combined with previous research results indicating elevation in C5a, would seem to indicate that formation of the MAC accompanies strenuous exercise. The formation of the MAC following strenuous exercise could ultimately serve to destroy and assist in clearing the by-products of proteolysis and/or haemolysis.

Factor B, a C3 convertase enzyme, was significantly elevated 72h after the race (Fig. 3). Dufaux and Order [8] proposed that the classical pathway of complement was activated following prolonged exercise. C3b deposited by the classical pathway can bind to Factor B, the enzyme involved in activating the alternate pathway of complement.[15,23] Thus it seems tenable that the alternate pathway as well as the classical pathway (initiated by CRP binding to debris/endotoxins) are initiated following strenuous exercise.

RF was significantly elevated 72h after the race (Fig. 4). Although RF peaked IPE no significance was reported, possibly due to the large subject variation at this measurement. Interestingly, as with CRP, RF was consistently elevated above the normal ranges. RF (IgM anti-IgG autoantibody) initiates inflammatory processes and is specific for a determinant on the Fc portion of the subjects' own IgG molecules. The complexes formed between RF and IgG are deposited in the synovia of joint spaces where they activate complement pathways and thus chemotactic factors that attract granulocytes.[15] The elevated RF at 72h after the race would imply that the immune/inflammatory response to the strenuous exercise had not yet resolved. This should be taken into consideration when monitoring and interpreting changes in immunoglobulin (IgG and IgM) levels following exercise.

The present study showed no significant alterations in C1est, C3 and C4. Studies have consistently yielded inconsistent results when it comes to monitoring the alterations in complement concentration following exercise. In a study conducted by Dufaux and Order,[8] 2.5 hours of running was adequate to elicit significant changes in C3a and C4a anaphylatoxins, the active components of C3 and C4 respectively. These changes were observed in moderately trained athletes. A similar response of C3a and C4a was shown in another study by Dufaux *et al*.[9] where the concentration of these complement components was significantly elevated immediately following a graded cycle ergometer test to exhaustion. In addition, Camus *et al*.[2] found a significant increase in C5a following 20 minutes of cycling at 80% of VO_{2max}. However, Thomsen *et al.*[28] showed no significant changes in complement cleaved products (C3c and C3d) in 14 untrained volunteers who completed a 60 minute cycle test at 75% of VO_{2max}. It was hypothesised by Camus *et al.*[2] that previous studies had failed to show changes in C5a due to the nature of the intervention (exercise and intensity) and also the turnover rate of this protein. It is tenable that this hypothesis could apply to the present study as within 3h post exercise, C1est, C3 and C4 were below pre-race levels. Clearly, the conflicting results with regard to complement response following exercise requires further elucidation. When comparing and interpreting the complement response to exercise, the mode, intensity, duration of the activity as well as experience of the athletes should be taken into consideration. A study conducted by King *et al*.[16] emphasises this point as it was found that certain activities such as jogging and aerobic dancing were characterised by a decreased possibility of elevated inflammatory markers. Although the study did not take into account the duration and intensity of the exercise, activities such as swimming, cycling and weight -lifting did not exhibit the same association. In addition, Nieman *et al.*[19] have shown that resting as well as post-exercise levels of complement differ in athletes and sedentary controls.

No significant alterations in circulating immune complexes were found in the present study. Soluble antigens form antibody:antigen complexes known as immune complexes.[23]

Thus each time antigen meets an antibody, an immune complex is formed.[15] These antigens include (amongst others) debris from dead micro-organisms and are removed from circulation via complement activation. One might expect that damage caused as a result of prolonged, strenuous exercise to muscles, connective tissue and erythrocytes results in the formation of excessive immune complexes. The results, however, show that CIC were consistently below the normal range following the ultramarathon (Table I). Although CIC are not strictly classified as acute phase reactants, they can be seen as residual products of an APR and could thus provide information regarding the effectiveness of complement pathways to mediate their removal.[24] The non-significant changes in CIC could arguably be attributed to the efficient functioning of macrophages in rapidly removing the immune complexes from circulation and would be in keeping with elevated levels of CRP. This could also explain why little changes in complement concentrations were observed.

In summary, this study showed that CRP was significantly elevated immediately after an ultramarathon (90 km) and peaked 24h post marathon. CRP and RF were elevated above the normal ranges both at baseline and at subsequent measurements. The raised resting levels of CRP could imply that the athletes were training too close to the race, and that correct tapering was not implemented. Since certain APP were only elevated at 72h it is proposed that future studies should measure APP over a more extended period and at shorter intervals to obtain a comprehensive overview of the acute phase inflammatory response to exercise.

References

1. Benjamini E, Sunshine G, Leskowitz S. Complement. In: *Immunology: A Short Course*. III. New York: Wiley-Liss, 1996: 245-59.
2. Camus G, Duchateau J, Dupont-Deby G, *et al*. Anaphylatoxin C5a production during short-term submaximal dynamic exercise in man. *Int J Sports Med* 1994; **15**(1): 32-5.
3. Cannon JG, Meydani SN, Fielding RA, *et al*. Acute phase response in exercise II. Associations between vitamin E, cytokines and muscle proteolysis. *Am J Physiol* 1991; **260:** 1235-9.
4. Deschenes MR, Brewer RE, Bush JA, McCoy RW, Volek JS, Kraemer WJ. Neuromuscular disturbance outlasts other symptoms of exercise-induced muscle damage. *J Neurol Sci* 2000; **174:** 92-9.
5. Dill DB, Costill DL. Calculation of percentage changes of blood, plasma and red cells in dehydration. *J Appl Physiol* 1974; **37:** 247-8.
6. Dinarello CA. Interleukin-1 and the pathogenesis of the acute-phase response. *N Engl J Med* 1984; **311:** 1413-8.
7. Du Clos TW. Function of C-reactive protein. *Ann Med* 2000; **32:** 274-8.
8. Dufaux B, Order U. Complement activation after prolonged exercise. *Clin Chim Acta* 1989; **179:** 45-50
9. Dufaux B, Order U, Liesen H. Effect of short maximal physical exercise on coagulation, fibrinolysis and complement. *Int J Sports Med* 1991; **12:** 38-42.
10. Epstein FH. Acute phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340:** 448-54.
11. Fallon KE. The acute phase response and exercise: The ultra-marathon as prototype exercise. *Clin J Sport Med* 2001; **11:** 38-43.
12. Fallon KE, Fallon SK, Boston T. The acute phase response and exercise: court and field sports. *Br J Sports Med* 2001; **35:** 170-3.
13. Fridén J, Sjostrom M, Ekblom B. Myofibrillar damage following intense exercise in man. *Int J Sports Med* 1983; **4:** 170-6.
14. Gleeson M, Almey J, Brooks S, Cave R, Lewis A, Griffiths H. Haematological and acute-phase responses associated with delayed-onset muscle soreness in humans. *Eur J Appl Physiol* 1995; **71:** 137-42.
15. Janeway CA, Travers P, Walport M, Shlomchik M. *Immunobiology. The Immune System in Health and Disease*. 5th ed. New York: Garland Publishing, 2001.
16. King DE, Carek P, Mainous AG, Pearson WS. Inflammatory markers and exercise: Differences related to exercise type. *Med Sci Sports Exerc* 2003; **35:** 575-81.
17. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med* 2000; **21:** 21-4.
18. Meyer T, Gabriel HW, Ratz M, Muller HJ, Kindermann W. Anaerobic exercise induces moderate acute phase response. *Med Sci Sports Exerc* 2000; **33:** 549-55.
19. Nieman DC, Tan SA, Lee JW, Berk LS. Complement and immunoglobulin levels in athletes and sedentary controls. *Int J Sports Med* 1989; **10:** 124-8.
20. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv Immunol* 1983; **34:** 141-99.
21. Peters EM, Anderson R, Theron AJ. Attenuation of increase in circulating cortisol and enhancement of the acute phase protein response in vitamin C-supplemented ultramarathoners. *Int J Sports Med* 2001; **22:** 120-6.
22. Petibois C, Cazorla G, Déléris G. The biological and metabolic adaptations to 12 months training in elite rowers. *Int J Sports Med* 2003; **24:** 36-42.
23. Roitt I, Brostoff J, Male D. *Immunology*. 6th ed. Edinburgh: Mosby, 2002.
24. Schifferli JA, Taylor RP. Physiological and pathological aspects of circulating immune complexes. *Kidney Int* 1989; **35:** 993-1003.
25. Schultz DR, Arnold PI. Properties of four acute phase proteins: C-reactive protein, serum amyloid A, (1-acid glycoprotein, and fibrinogen. *Semin Arthritis Rheum* 1990; **20:** 129-47.
26. Strachan AF, Noakes TD, Kotzenberg G, et al. C-reactive protein concentrations during long distance running. *BMJ* 1984; **29:** 1249-51.
27. Taylor C, Rogers G, Goodman C, *et al*. Hematologic, iron-related, and acute-phase protein responses to sustained strenuous exercise. *J Appl Physiol* 1987; **62:** 464-9.
28. Thomsen BS, Rodgaard A, Tvede N, *et al*. Levels of complement receptor type one (CR1, CD35) on erythrocytes, circulating immune complexes and complement C3 split products C3d and C3c are not changed by short term physical exercise or training. *Int J Sports Med* 1992; **13:** 172-5.
29. Tomaszewski M, Charchar FJ, Przybycin M, *et al*. Strikingly low circulating CRP concentrations in ultra-marathon runners independent markers of adiposity. How low can you go? *Arterioscler Thromb Vasc Biol* 2003; **23:** 1640-4.
30. Weight LM, Alexander D, Jacobs P. Strenuous exercise analogous to the acute-phase response? *Clin Sci* 1991; **81:** 677-83.