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The Acute Phase Response and Exercise: The Ultramarathon as Prototype Exercise

Kieran E. Fallon, MBBS, MSc

Department of Sports Medicine, Australian Institute of Sport, Belconen, Australian Capital Territory, Australia

Objective: Controversy exists in relation to the nature of the acute phase response, which is known to occur following endurance exercise. This study was conducted to demonstrate the similarities between this response and the response consequent to general medical and surgical conditions.

Design: This is a case series field study of serum levels of acute phase reactants in a group of ultramarathon runners competing in a 6-day track race.

Participants: Seven male and one female experienced ultramarathon runners.

Intervention: A track race of 6 days duration.

Main Outcome Measures: Serum iron, ferritin, transferrin, albumin, haptoglobin, alpha-1 antitrypsin, complement components 3 and 4, C-reactive protein, and erythrocyte sedimentation rate, total iron binding capacity, and transferrin saturation.

Results: Of the 11 acute phase reactants measured, 6 (serum iron, ferritin, percent transferrin saturation, C-reactive protein, erythrocyte sedimentation rate, and haptoglobin) responded as if an acute phase response was present; 5 (tranferrin, albumin, alpha-1 antitrypsin, and complement components 3 and 4) did not respond in such a fashion.

Conclusion: This study provides further evidence that the acute phase response consequent to exercise is analogous to that which occurs in general medical and surgical conditions. The previous demonstration of the presence of the appropriate cytokines following exercise, the findings of others in relation to acute phase reactants not the subjects of this study, the possibility that a training effect leading to attenuation of the response and the realization that the acute phase response is not identical across a range of medical conditions lends weight to the above conclusion.

Key Words: Acute phase response—Iron—Plasma proteins—Inflammation.

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INTRODUCTION

Considerable controversy exists as to whether the hematological and biochemical response to exercise is analogous to the acute phase response (APR) that occurs in a number of disease states.

The APR occurs following a variety of tissue insults including bacterial infection, surgery, burns, neoplasia, tissue infarction, and in inflammatory diseases.[1] The response has a number of components including increases in serum concentrations of hormones, metabolic changes including increased protein catabolism and negative nitrogen balance, changes in lipid metabolism, alterations in serum concentrations of cations, changes in iron metabolism, leukocytosis, complement activation, and increases in plasma proteins, which are primarily produced in the liver.[2] In contrast to the increases in some plasma proteins, concentrations of albumin, transthyretin, alpha fetoprotein , alpha-2 HS glycoprotein, and transferrin fall during the response, and these are designated as negative acute phase reactants.[1] Inflammation such as that associated with exercise is also associated with abnormalities of iron metabolism including increased tissue storage of iron (increased serum ferritin), decreased serum iron, total iron-binding capacity (TIBC), transferrin, and transferrin saturation.[3,4]

It has been proposed that the APR to exercise is related to damage to skeletal muscle, which is typically suggested by increases in serum creatine phosphokinase (CPK).[5] A number of studies have documented aspects of the APR following events that would be expected to induce significant damage to skeletal muscle.[6–11] These studies, two of which quantitate muscle trauma by measurement of CPK,[6,9] provide contradictory evidence for the presence of an APR occurring in response to prolonged exercise. Possible explanations for this confusion may be failure to take plasma volume changes into account, lack of appreciation of the time course of components of the APR, and the heterogeneous nature of the APR to other stressful events. In addition the presence of concomitant processes such as plasma volume expansion and hemolysis, which may mask changes in albumin and haptoglobin, respectively, also need to be taken into account.

From a clinical perspective, documentation of the extent and nature of the APR to various types of exercise is important, as changes related to the response may have to be accounted for during interpretation of hematological

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Address correspondence and reprint requests to Kieran E. Fallon, MBBS, MSc, Department of Sports Medicine, Australian Institute of Sport, P.O. Box 176, Belconnen ACT 2616, Australia.

and biochemical measurements taken during and after participation in sport.

The aim of this study was therefore to investigate, in detail, the APR to very prolonged exercise, which, based on CPK values, is known to induce significant muscle damage. It was hypothesized that this extreme form of exercise would be likely to induce a greater APR than had been previously documented, and that the question of the similarity between the response to exercise and other physiological insults would be resolved.

METHODS

Prior to commencement of this study, approval was obtained from the Ethics Committee of the Australian Institute of Sport. All procedures conformed to the National Health and Medical Research Council guidelines for experimentation with human subjects, and all subjects gave their informed written consent prior to participation in the study. All potential participants were contacted and, of a total of 18 entrants, 16 of whom qualified for inclusion, 11 experienced ultramarathon runners volunteered as subjects for this study. One male was excluded due to the presence of iron deficiency anemia, and two of the subjects failed to complete the event. The exclusion criteria were 1) the presence of the following conditions at the start of the race: orthopedic injury, infection, acute or chronic inflammatory disease, and iron-related disorders, 2) use of antiinflammatory or iron-containing medication, 3) blood loss, including menstruation during the event, and 4) development of an infection or acute inflammatory disease during the event.

The data thus presented was derived from seven males and one female.

The 1996 Colac Six Day race was a continuous running event during which runners attempted to cover the greatest possible distance in 6 days. The event was conducted on a 400 m oval grass track, the surface of which changed to uneven dirt during the event. Mean maximum and minimum daily temperatures were 16°C and 10°C, respectively, and the mean daytime relative humidity was 60%. The runners changed direction every 2 hours.

Twenty milliliters (ml) of blood was drawn from an antecubital vein, using a sterile technique, 30 minutes prior to race start at 1500 hours. Further samples were obtained at 0900 daily, and a final sample was obtained within 15 minutes of the conclusion of the event at 1500 hours on day 6. To minimize the effect of positional change on plasma volume, each sample was obtained immediately after the runner assumed a sitting position. Following centrifugation and processing, specimens were packed in refrigerated containers and transported to a central laboratory where they were analyzed within 18 hours of collection.

Measurements of serum iron, transferrin, ferritin, total iron binding capacity, percent transferrin saturation, albumin, creatine phosphokinase, and haptoglobin were performed on a Hitachi 911 analyzer (Roche Diagnostics, Basel, Switzerland) using Boehringer Mannheim reagents. C-reactive protein (CRP), alpha-1 antitrypsin, and complement components 3 and 4 were measured by nephelometry on a Beckmann Array Protein System (Brea, CA, U.S.A.) using Beckmann reagents. The erythrocyte sedimentation rate (ESR) was measured using standard manual methods.

The particular biochemical variables chosen are representative of acute phase reactants with different time courses and degrees of response. Some were included, as they had not been previously studied in an event of this type.

All concentrations were corrected for changes in plasma volume, which were calculated by the method of Dill and Costill.[12] Plasma volume changes were calculated individually for each sample on each day.

All dependent variables were analyzed using a repeated measures one way analysis of variance (ANOVA) with Neumann-Keuls post hoc procedures. Due to the extreme variability observed in the response creatine phosphokinase (CPK) (MSE > 64,000,000), a nonparametric Wilcoxon matched pairs test was used to evaluate whether changes from baseline were significantly different on each morning ($p < 0.05$). Statistical analysis was performed using Statistica, version 5.1, StatSoft Inc, Tulsa, OK, U.S.A.).

RESULTS

Subject characteristics and distances covered are shown in Table 1. All participants were Caucasian. One runner had a history of borderline hypertension, which was not under pharmacological management, and one had a history of a lumbar disc lesion. All previously had had running-related injury to the lower limbs but none had an injury at the commencement of the event.

Findings for iron-related parameters are summarized in Table 2. There was a significant main effect for a reduction in serum iron ($p = 0.0003$) but only postrace was the value significantly lower than baseline (baseline 17.3 μmol/L versus postrace 6.0 μmol/L). There was a significant main effect for an increase in serum ferritin ($p < 0.00001$), the value being significantly higher than baseline (117 ng/ml) on days 2 (194 ng/ml), 3 (261 ng/ml), 4 (251 ng/ml), 5 (243 ng/ml), 6 (237 ng/ml), and postrace (246 ng/ml), ($p < 0.005$). There was a significant main effect for a decrease in TIBC ($p = 0.000002$),

TABLE 1. *Subject characteristics (mean/standard deviation)*

Age	47	7
Years of running	15	8
Weekly training distance (km)	161	51
Previous ultramarathons	21	16
Previous marathons	17	15
Best 6-day distance (km)	714	153
Best marathon time (hrs)	2.97	0.4
Weight	70.6	8.2
Running Distance (km)		
Day 1	142.6	28.8
Day 2	103.3	28.3
Day 3	93	28.0
Day 4	97.9	26.0
Day 5	92.3	31.0
Day 6	93.1	27.6

TABLE 2. *Iron-related parameters (means and standard deviations)*

	Prerace	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Postrace
Iron (μmol/L)	17.3 (6.2)	10.4 (4.1)	23.5 (17.4)	15.8 (7.2)	13.5 (8.5)	10.9 (3.8)	7.7 (2.3)	6.0 (1.5)*
Ferritin (ng/ml)	117 (111)	150 (117)	194 (85)**	261 (134)**	251 (115)**	243 (109)**	237 (99)**	246 (114)**
Transferrin (g/L)	3.09 (0.45)	3.07 (0.50)	2.96 (0.51)	3.01 (0.51)	3.00 (0.52)	2.90 (0.44)	3.04 (0.54)	3.11 (0.47)
TIBC (μmol/L)	42 (14)	45 (15)	52 (18)	38 (17)	31 (9)*	51 (9)	50 (9)	52 (8)
Transferrin saturation (%)	47 (22)	24 (9)*	36 (17)	43 (15)	43 (20)	22 (8)*	16 (7)**	12 (3)**

* $p < 0.05$, ** $p < 0.005$.
TIBC, total iron binding capacity.

but only the day 4 value (31 μmol/L) was significantly lower than baseline (42 μmol/L) ($p < 0.05$). There was a significant main effect for a reduction in percent transferrin saturation ($p = 0.000008$) with values significantly lower than baseline (47%) on days 1 (24%) and 5 (22%) ($p < 0.05$) and on day 6 (16%) and postrace (12%) ($p < 0.005$).

There were no significant changes in serum transferrin concentration (Table 3).

There was a significant main effect for an increase in serum CRP ($p = 0.000001$), and on all days and postrace the concentrations were significantly elevated above baseline ($p < 0.05$). There was a significant main effect for an increase in ESR ($p = 0.002$) but only postrace was the value significantly higher than baseline (baseline, 3 versus 15 postrace, $p < 0.05$). There was a significant main effect for serum haptoglobin ($p < 0.000001$), with a significant decrease occurring on day 1 ($p < 0.02$), and significant elevations occurring on day 3 ($p < 0.05$) and days 4–6 and postrace ($p < 0.0005$). There were no statistically significant changes in C3, C4, albumin, and alpha-1 antitrypsin (AAT) (Table 4).

CPK was significantly elevated above baseline on all days and postrace.

DISCUSSION

This study provides further evidence in support of the hypothesis that the APR to endurance exercise is analogous to that which occurs following other tissue insults. Of the 11 acute phase reactants measured, 6 (serum iron, ferritin, percent transferrin saturation, CRP, ESR, and haptoglobin) responded as if an APR was present. Five did not respond in such a fashion, but a possible explanation for this behavior is suggested for three of these. The findings are in some cases consistent with, and in others different to, those previously found in studies of endurance exercise and the APR.

While CPK is not a reliable indicator of the extent of muscle damage, the large increases in this enzyme indicate that significant muscle damage or membrane leakage occurred during this event. As it has been postulated that muscle damage and inflammation is the process underlying the APR to exercise, it is proposed that this event provided a sufficient stimulus for this response to occur.

A number of authors have previously demonstrated aspects of the APR in the context of endurance exercise. Immediately following a marathon, Weight et al. demonstrated increases in white cell count (WCC) and CRP but also found increased albumin and decreased haptoglobin. Twenty-four hours later, haptoglobin was significantly increased, as was fibrinogen,[6] the earlier decrease in haptoglobin probably being related to hemolysis. It was concluded that the response to sustained exercise was similar to but not analogous to the APR.

In response to running 25 km/day[1] for 4 days, Dufaux et al. found an increase in CRP on days 3 and 5 but no change in C3 and C4.[7] Immediately after a 100 km run, Poortmans and Haralambie found increased albumin, transferrin, and alpha-1 glycoprotein and decreased haptoglobin, and on the following day a persistent decrease in haptoglobin and increases in alpha-1 glycoprotein and AAT.[8] Again these responses are not totally consistent with the APR; however, the lack of correction for potential plasma volume changes, the occurrence of acute hemolysis, and changes in albumin related to plasma volume changes may partially explain these findings.

Taylor et al. assessed the response to a 160 km triathlon. Immediately after and 30 minutes after the event, an increase was found in WCC, and serum iron and trans-

TABLE 3. *Other acute phase reactants*

	Prerace	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Postrace
CRP (mg/dL)	0.19 (0.14)	2.19 (1.29)**	3.75 (1.70)**	2.79 (1.73)**	2.55 (2.12)**	1.83 (1.15)**	1.76 (0.84)**	1.84 (0.88)**
ESR	3 (1)	5 (7)	5 (3)	10 (13)	10 (6)	9 (4)	11 (4)	15 (7)**
C3 (g/L)	1.24 (0.13)	1.33 (0.24)	1.12 (0.21)	1.10 (0.25)	1.18 (0.39)		1.16 (0.30)	1.09 (0.28)
C4 (g/L)	0.23 (0.05)	0.24 (0.08)	0.22 (0.05)	0.22 (0.05)	0.24 (0.07)		0.22 (0.07)	0.23 (0.07)
Albumin (g/L)	50 (4)	51 (6)	52 (8)	54 (7)	52 (8)	52 (8)	53 (8)	56 (7)
Haptoglobin	1.06 (0.21)	0.61 (0.42)*	1.18 (0.45)	1.49 (0.41)*	1.74 (0.42)**	2.03 (0.42)**	1.99 (0.42)**	2.06 (0.50)**
Alpha-1 antitrypsin (g/L)	1.39 (0.22)	1.74 (0.53)	1.56 (0.24)	1.64 (0.34)	1.84 (0.51)		1.75 (0.34)	1.81 (0.41)

* $p < 0.05$, ** $p < 0.005$.
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

TABLE 4. *Creatine phosphokinase (μ/L) (mean and standard deviation)*

	Prerace	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Postrace
Mean	102	9,880*	9,243*	12,240*	7,335*	4,930*	4,916*	4,826*
SD	29	15,305	7,845	21,347	12,987	7,667	7,136	6,603

* $p = 0.01$.
SD, standard deviation.

ferrin were decreased. At 24 hours these parameters had returned to baseline but CRP was elevated. At 48 hours all parameters had returned to baseline.[9] At 48 hours following a 160 km run, Dickson et al. found a significant increase in serum ferritin and no change in haptoglobin,[10] and in a study based on iron-related parameters in which runners covered 50 km per day for 20 days, no change was found in serum iron, % transferrin saturation, ferritin, and transferrin at the conclusion of the event.[11]

The reduction of serum iron found in our study has been previously demonstrated during and after a 1,600 km ultramarathon,[13] following a 24-hour race,[14] a 20-day cycle tour,[15] and a 160 km triathlon,[9] whereas no change was found following a 42 km marathon[6] and a 1,000 km run in which 50 km was covered on each of 20 days.[11] The authors of the last mentioned study indicated that wide variability in the iron response may have led to the lack of significance of the change in iron levels.

The increase in serum ferritin has been previously demonstrated during and after a 1,600 km ultramarathon,[13] following a 24-hour race,[14] a 20-day cycle tour,[15] and 48 hours after a 160 km run.[10] A nonsignificant 40% increase was seen following a 160 km triathlon.[9] Again no change was found following a 42 km marathon[6] and a 1,000 km run in which 50 km was covered on each of 20 days.[11]

The reduction in transferrin saturation has been previously demonstrated during and after a 1,600 km ultramarathon,[13] following a 160 km triathlon,[9] but was not found during a 20-day cycle tour,[15] a 1,000 km run in which 50 km was covered on each of 20 days,[11] and following a 42-km marathon.[6]

The increase in haptoglobin has been previously demonstrated during and after a 1,600 km ultramarathon[13] and 48 hours after a marathon.[6] Other studies demonstrated no change 48 hours after a 160 km race,[10] half an hour after a 160 km triathlon,[9] and 24 hours after a 24 hour run.[14] Haptoglobin levels are reduced following exercise during which hemolysis occurs, and should the serum concentration be measured in the period during which hemolysis is occurring and before the APR is fully operative, both reduced and/or normal levels may be detected. In our study the haptoglobin level was reduced on day 1, not significantly different to prerace levels on day 2, and was elevated thereafter.

The increase in CRP has been previously demonstrated following a 160 km triathlon[9] and a 42 km marathon,[6] and a distance-related increase was demonstrated over 15–88 km of running by Strachan.[16]

Changes in ESR appear not to have been studied in relation to endurance exercise. Despite increases in mean levels of greater than 300% during the 6-day event, a statistically significant increase was seen only immediately following the finish. The ESR is related to fibrinogen, and this parameter was measured following a marathon run. A significant increase was found at 1, 2, and 6 days following that event.[6]

The response of five parameters was not consistent with the APR.

Serum transferrin, a negative acute phase reactant, did not change at any time during the run. The related total iron-binding capacity was also unchanged except for a reduction on day 4, a response similar to that which occurred during a 1,600 km run.[13] Transferrin concentration was reduced following the 1,000 km stage race studied by Seiler,[11] but the change was not statistically significant.

Serum albumin, a negative acute phase reactant, did not change at any time during the run. Weight[6] found an increase immediately after a marathon, but no change from prerace to over the ensuing 6 days. Any decrease in this parameter related to an APR is likely to be masked by the changes related to the concurrent increase in plasma volume (8–22%) that occurred following day 1 of the run. A net influx of proteins into the intravascular space occurs when plasma volume is expanded, and therefore no change in serum albumin would be expected. In fact no change was found in albumin concentration on day 4, and increased concentration was found on day 11 and immediately following a 16-day run.[17]

Serum concentrations of complement components 3 and 4 typically increase during the APR. No change in either component was found in this study. Defaux[7] reported increases in both C3 and C4 following four days of running 25 km a day, but these changes were related to changes in total serum protein and do not appear to have been significant when absolute values were compared. No change in C3 and C4 was found following a 6.6 km run.[18] In contrast Defaux[19] found increases in C3a and C4a following a 2.5-hour running test. Explanation for the absence of change in serum levels of complement components may lie in the dynamic nature of the complement system in which C3 and C4 are converted to C3a and C4a. As with most serum concentrations, the concentration detected is the result of both production and clearance, and therefore unchanging concentrations do not necessarily indicate that increased production is absent.

While serum concentrations of alpha-1 antitrypsin (A1A) increased by up to 32% during the event, the changes were not statistically significant. In contrast small elevations were found, which persisted for several

days following 2- and 3-hour runs,[19] and on the day following a 100 km run.[8] In a prolonged training study, 13 elite swimmers were followed during 20 days of heavy training. A significant 25% increase in A1A was detected by day 19, and this persisted for 21 days following the end of the training period.[21]

Despite the number of studies conducted, the complete set of responses that accompany the APR to tissue injury has not been assessed in the context of short duration or prolonged exercise, and no single study has provided evidence of an APR in all parameters under assessment. In our study we could offer no explanation for the lack of response in serum transferrin and alpha-1 antitrypsin.

This, however, is insufficient reason to conclude that exercise does not induce an APR that is mechanistically equivalent but in some ways different to that consequent upon other threats to homeostasis. The APR to various medical conditions is not absolutely equivalent. Baynes et al.[4] assessed the response of lactoferrin, total white cells, CRP, serum ferritin, serum iron, TIBC, transferrin, and transferrin saturation in acute lobar pneumonia, active pulmonary tuberculosis, rheumatoid arthritis patients undergoing gold therapy, and sepsis in immunosuppressed patients. At the onset of the study, differences across the groups were found in lactoferrin, total white cell count, serum iron, TIBC, and % transferrin saturation. During therapy, differences across the groups were found in lactoferrin, total white cell count, CRP, ferritin, and TIBC. It is therefore not surprising that differences may be found in the acute response to exercise.

In addition, other studies have demonstrated changes in other parameters consistent with the APR. An increased white cell count and relative and absolute neutrophilia accompanied by an increase in platelets was found during a 1,600 km run.[13] The leukocytosis of exercise has been demonstrated over a wide range of exercise duration, but is not always related to an APR.[22] Liesen et al.[20] demonstrated increases in alpha-1 acid glycoprotein, ceruloplasmin, and plasminogen following several hours of running.

The effect of training on the APR also requires consideration. In the only study of its type, Leisen[20] studied the changes in the APR to 2 hours of running before and after 9 weeks of endurance training. A decrease in the response of CRP, haptoglobin, and alpha-1 acid glycoprotein was demonstrated following training. In addition Leisen found that well-trained athletes have elevated levels of alpha-1 antitrypsin, alpha-2 macroglobulin, and C1 inhibitor, which may, as an adaptive response, assist in limiting the exercise-induced inflammatory response and perhaps the APR.

Studies of the mechanisms underlying the APR also support the concept of an equivalent ARP following exercise. The cytokines responsible for the major manifestations of the APR, interleukin (IL)-1, interleukin-6, and tumor necrosis factor (TNF) have been shown to be elevated during and after prolonged exercise. TNF was found to be elevated 1, 3, and 24 hours following a 2.5-hour run,[23] IL-6 and IL-1 were elevated following a marathon,[24] and IL-6 following a 20 km race.[25] In addition there is some evidence that the cytokine response, particularly that of IL-6, is much greater following eccentric exercise,[26] and this may explain some of the disparate results in the exercise APR studies.

The action of cytokines is complex, and cytokine combinations have unpredictable effects.[27] The effects of combinations of cytokines in varying concentrations and sequences of production are areas of ongoing research. The effects of cytokines are influenced by hormones such as insulin, receptors and antagonists, autoantibodies, and binding to plasma proteins, and therefore their actions must be seen in the context of the cell injury that occurs during exercise. This may also explain the differences in the APR associated with exercise.

In summary, this study provides further evidence that the APR consequent to exercise is analogous to that which occurs in general medical and surgical conditions. The previous demonstration of the presence of the appropriate cytokines following exercise, the findings of others in relation to acute phase reactants not the subjects of this study, the possibility of a training effect leading to attenuation of the response, and the realization that the APR is not identical across a range of medical conditions lends weight to the above conclusion.

There is clear need for a study in which a significant exercise stress is combined with measurements of TNF, IL-6, and IL-1 and a wide range of acute phase reactants.

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