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ORIGINAL ARTICLE

Biomarkers of muscle and cartilage damage and inflammation during a 200 km run

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Abstract Ultra-marathon running is frequently associated with muscle fibre damage. However, ultra-marathon related information is scarce. The present study evaluated muscle and cartilage biomarkers, and cytokine secretion during a 200 km running event. Venous blood samples from 54 trained male ultra-marathon runners (mean ± SD, 45.7 ± 5.1 years). Plasma creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate, glucose, high-sensitivity C-reactive protein (Hs-CRP), interleukin-6 (IL-6), TNF-∝ and serum cartilage oligomeric matrix protein (COMP) content were determined before, midway and immediately after the race. CPK increased 90-fold (19-fold at 100 km) from pre-race value and LDH increased 3.7-fold (2.2-fold at 100 km). AST increased 15-fold (5-fold at 100 km) and ALT increased 3.9-fold (2-fold at 100 km). Blood lactate and glucose levels did not change significantly. Hs-CRP increased 23-fold (3-fold at 100 km) and IL-6 increased 121-fold at 100 km, and then remained stable up to 200 km, whereas TNF-∝ did not change significantly. Serum COMP increased 3-fold (1.3-fold at 100 km). Post-run CPK was correlated with LDH ($r = 0.62$, $P < 0.001$), Hs-CRP ($r = 0.45$, $P < 0.001$), ALT ($r = 0.89$, $P < 0.001$), AST ($r = 0.97$, $P < 0.001$), and IL-6 ($r = 0.61$, $P < 0.001$). The present study demonstrated that blood biomarkers related to muscle and cartilage damage and inflammation were increased during a 200 km run and that this was particularly marked during the second half of the event. Ultra-marathon running clearly has a major impact on muscle and cartilage structures.

Keywords Ultramarathon · Cartilage damage · Muscle inflammation · COMP · Hs-CRP

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Introduction

Ultra-endurance races, held typically over 200 km, are increasingly popular but no information is available on its effects on muscle and cartilage integrity. Endurance exercise induces numerous cellular changes in the body such as deterioration in the structure and function of muscle (Kuipers 1994), cartilage (Kersting et al. 2005; Muendermann et al. 2005; Neidhart et al. 2000; Wong and Carter 2003) and liver (Fallon et al. 1999; Negal et al. 1990), as well as major increases in cytokines (Nieman et al. 2001, 2005; Suzuki et al. 2000).

Plasma creatine phosphokinase (CPK) activity is widely used as a marker of muscle damage during exercise. Noakes (1987) suggested that increase in plasma CPK is related to the duration and intensity of exercise. Cartilage oligomeric matrix protein (COMP) has been suggested as a biomarker that is responsible for cartilage damage as a result of acute exercise (Kersting et al. 2005). Eckstein et al. (2004) reported that more intense loading during exercise leads to a greater knee cartilage deformation and that running is the most stressing type of exercise on patella cartilage when compared to cycling, walking, squatting, and knee bending.

Strenuous exercise induces increased levels in a number of pro- and anti-inflammatory cytokines. Nieman et al. (2005) reported that muscle CPK release and DOMS (delayed onset of muscle soreness), after a 160 km run, is related to changes in cytokines. Such changes in biomarkers, related to muscle and cartilage damage during ultra-endurance exercise, have been interpreted as showing an acute immunological response related to muscle damage.

The determination of the changes in biochemical variables, such as these, during a 200 km running may help in the selection of an appropriate upper limit in the distance of races to limit injuries for participants in future long-distance competition. The aim of this study was to evaluate biochemical changes related to muscle and cartilage damage and inflammation during a 200 km ultra-marathon race. We considered that damage to both muscle and cartilage would be related to the distance of the race.

Methods

Subjects and race description

The blood samples were obtained before, midway (100 km) and at the end of a 200 km race held in Cheju Island (at sea level) in South Korea from 54 males who were experienced ultra-marathon runners aged 38–59 years. One hundred thirty runners participated in the race and 27 (20%) dropped out during the race. All three blood samples were obtained from only 54 subjects because of the short interval between runners. Mean body weight and height were 68 kg (54–73) and 170 cm (165–182) and all subjects had been trained for more than 3 (3–10 years) years. The runners performed the event with a cut-off time of 36 h (actual performance times ranged from 23:53 to 34:56). The temperature during the race ranged from −1 to 5°C (mean 1.8°C), with relative humidity between 59 and 78% and wind speed 3.1–3.2 km/h. The runners were provided with food and drink ad libitum. The experimental procedures for this study were approved by the Ethics Committees of Korea National Sport University and the subjects provided with both oral and written information about the experimental procedures, before their written informed consent was obtained (Table 1).

Table 1 Subject characteristics (n = 54)

Variables	Mean (SD)	Range
Age (year)	45.7 (5.1)	35–59
Height (m)	1.73 (0.5)	1.60–1.80
Body fat (%)	18.6 (4.4)	4.7–23.3
Running history (years)	7.8 (4.5)	3–21
Ultra-marathon races (number)	15 (5.6)	3–25
Pre-race body mass (kg)	68.8 (5.9)	57.1–80.8
Post-race body mass (kg)	66.3 (6.3)	55.8–81.1
Race time (h)	32.1 (3.5)	22.5–34.5

Blood sampling and analysis

Eight milliliters of blood was drawn from an anticubital vein during registration at 10–12 h before the race, immediately after 100 km, and at the end of 200 km run. The blood was placed into tubes containing EDTA (1 mg/ml), or dry tubes for separating serum. Samples were stored at −80°C after centrifugation (3,000 rpm for 15 min). Plasma volume changes were estimated by hematocrit according to Dill and Costill (1974). All results were corrected for changes in plasma volume. Serum COMP (AnaMar Medical, Gotenborg, Sweden), plasma IL-6 and TNF alpha (R&D systems, Minneapolis, MN) concentrations were evaluated by enzyme-linked immunosorbent assays based on two monoclonal antibodies. Plasma levels of glucose, lactate, creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and high-sensitive C-reactive protein (Hs-CRP) were measured using an auto-analyzer (ADIVA 1650, Bayer, USA). All measurements were performed in duplicate (Table 2).

Statistical analysis

Variables are expressed as mean and standard deviation (SD). One-way analysis of variance (ANOVA) with repeated measures (pre-race, 100 and 200 km) were used to compare variables. Pearson product–moment correlation was used to test the relationship

Table 2 Blood variables at rest and during the event (n = 54)

Variables	Pre-race	100 km	200 km
LDH (IU/l)	279 (58)	609 (281)**	1,046 (532)**,†
Lactate (IU/l)	1.62 (0.54)	2.58 (1.13)	2.43 (0.9)
Glucose (mg/dl)	110 (39)	104 (25)	98 (45)
Hs-CRP (IU/l)	2 (4)	6 (6)*	46 (28)*,†
AST (IU/l)	24 (7)	121 (113)**	364 (282)**,†
ALT (IU/l)	23 (10)	40 (24)**	90 (52)**,†
IL-6 (pg/ml)	0.86 (0.17)	104.3 (45.5)†	108.6 (28.4)†
TNF-∝ (IU/l)	2.35 (1.56)	2.60 (1.43)	2.77 (1.82)
Hematocrit (%)	40.1 (4.32)	44.5 (10.53)	46.8 (12.7)

*P < 0.01 different from pre-race; **P < 0.001 different from pre-race; † P < 0.05 significant difference from 100 km; P < 0.001 significant difference from 100 km

between changes of CPK, COMP and measured biomarkers. The level of significance was accepted at a value of $P < 0.05$.

Results

Hemoconcentration induced by the 200 km run was about 10–15%. Blood glucose and lactate concentrations did not change during the race. AST and ALT were increased significantly at 100 km and a further rise was found at the end of the race. Mean values for AST increased 15-fold (5-fold at 100 km) and ALT increased 3.9-fold (2-fold at 100 km). LDH increased 3.7-fold (2.2-fold at 100 km). The CPK activity increased 90-fold (19-fold at 100 km) from the pre-race value at the end of the race. Change in CPK was significantly correlated with changes in plasma LDH ($r = 0.97$, $P < 0.001$), Hs-CRP ($r = 0.54$, $P = 0.001$), ALT ($r = 0.89$, $P = 0.001$), AST ($r = 0.97$, $P < 0.001$) and IL-6 ($r = 0.61$, $P < 0.001$), but not the race time, COMP and TNF-$\propto$.

A significant increase from pre-race level was recorded for IL-6 at 100 km (121×) but this was then unchanged between 100 and 200 km. Serum COMP was increased 3-fold (1.3-fold at 100 km). Hs-CRP was increased 23-fold (3-fold at 100 km) from the pre-race value. Mean values for TNF alpha were not significantly changed from the pre-race value during or at the end of 200 km race. Further significant increases in CPK, LDH, AST, and ALT, Hs-CRP, and COMP were found between 100 and 200 km.

Discussion

The results from this study demonstrate that a 200 km ultra-marathon race severely increases biomarkers related to muscle and cartilage damage, as well as the anti-inflammatory markers cytokine IL-6 and CRP. Furthermore, most of these inflammatory markers showed the greatest increase in the second half of the race (between 100 and 200 km).

Plasma CPK is a marker signifying muscle injury arising from myofibrillar disruption (Clarkson et al. 1992; Kuipers 1994; Noakes 1987). Fallon et al. (1999) and Nieman et al. (2005) showed elevated plasma CPK activity at the end of long-distance running. In our study, the CPK concentration increased much further in the second part of the race (Fig. 1). This result agreed with the suggestion of Noakes (1987) that the release of CPK is principally related to the duration of the exercise.

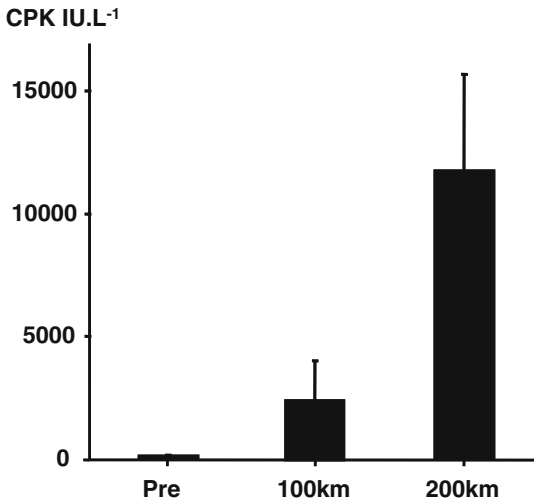

Fig. 1 Plasma CPK before and at the end of 100 and 200 km ultramarathon running

To our knowledge no previous study has examined the relationship between the duration of running and cartilage damage. COMP has been identified as an important constituent of the non-collagenous matrix within the hyaline cartilage (Kersting et al. 2005) and it was suggested as a biomarker for osteoarthritis and cartilage damage arising from acute exercise Fig. 2). Neidhart et al. (2000) showed that in experienced runners serum COMP was significantly increased during at least 3 weeks rest compared to sedentary controls. They also reported a significant rise in COMP after a marathon run. Muendermann et al. (2005) reported a COMP rise after 30 min of walking exercise, and Kersting et al. (2005) showed a correlation between cartilage volume loss after 1 h running and COMP concentration. Thus, it is likely that the 3-fold (1.3-fold at 100 km) increase in the serum COMP concentration, observed in the present study, represents a significant level of cartilage damage, especially in the second half of the race.

Intense physical exercise also induces acute-phase inflammatory reactions as demonstrated by the delayed increase of Hs-CRP from the liver. Hs-CRP release from hepatocytes is induced by IL-6 (Petersen and Pedersen 2005) and Hs-CRP has both a role in the induction of anti-inflammatory cytokines from circulating monocytes and in the suppression of the synthesis of pro-inflammatory cytokines from tissue macrophages (Pue et al. 1996). Pedersen and Hoffman-Goetz (2000) suggested that a greatest increase in plasma Hs-CRP is seen on the day after exercise of long duration. In our study, changes in plasma Hs-CRP occurred after 100 km and a further increase took place by the end of the race. Therefore, the 23-fold increase in Hs-CRP in

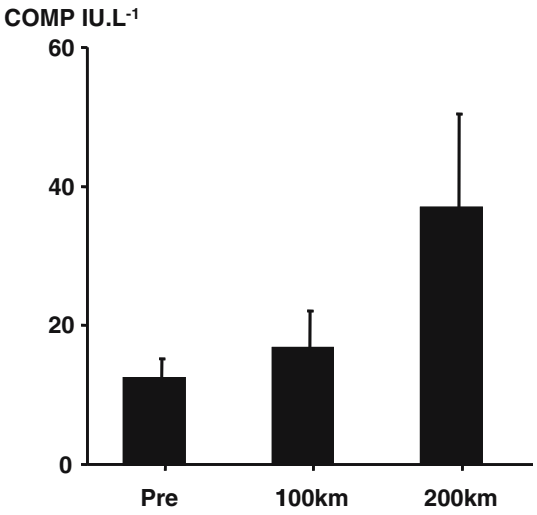

Fig. 2 Plasma COMP before and at the end of 100 and 200 km ultramarathon running

the latter half of the race may represent the synergistic effect of accelerated muscle or liver damage.

It is unclear whether hepatic damage occurs during ultra-marathon race as hepatic-marker enzymes, such as LDH, ALT, and AST, are found in both liver and muscle cells (Fallon et al. 1999). Therefore, increases in these enzymes do not specifically indicate liver cell damage although in runners they are used as general indicators of liver cell damage. In the present study, hepatic enzymes in plasma increased at 100 km, with a further increase in the second part of the race. The progressive increases in hepatic enzymes parallel the changes seen in studies of both shorter duration (Rehrer et al. 1992; Wu et al. 2004) and ultra-long distance races (1,000 km in 20 days) which report continuing hepatic and muscle cell injuries (Negal et al. 1990). Wu et al. (2004) reported biochemical data before and after a 24-h ultra-marathon race which was performed at sea level in Asian marathoners. Although the distance completed in Wu et al. (2004) study was shorter than the present study, the mean values of AST, ALT, and LDH after the race were in fact higher. The most likely explanation for the difference in the results from these studies is that this is the result of the effect of temperature difference. Noakes et al. (1987) suggested that the rise in serum enzyme activities after exercise is greater at altitude or in the heat, than after equivalent exercise at sea level or in the cold. Our present study was performed under the unfavorable environmental conditions such as low temperature (−1 to 5°C) and high wind speed (3.1–3.2 km/h).

IL-6 protein is released from skeletal muscle during prolonged exercise (Steenberg et al. 2000, 2001) and is involved in glucose homeostasis (Petersen and Perdersen 2005). In this study, an increase in IL-6 from pre-race level was measured at 100 km, and this increase remained during the second half of the race with a stable blood glucose concentration. The change of IL-6 in plasma is in accordance with the result of studies reporting a 40-fold increase immediately after a marathon race (Nieman et al. 2001) and increases 125-fold after 160 km-race (Nieman et al. 2005).

TNF-∝ has been proposed as the main cytokine inducer of acute phase reactions (Suzuki 2002). Two possibilities could explain the stable level of TNF-∝ expression during the 200 km race. The first is that the increase is related to exercise intensity. Strenuous exercise such as a marathon race (Ostrowski et al. 1998, 1999) and 50 km running (Mastaloudis et al. 2006; Starkie et al. 2001) induces an increase in the TNF-∝ concentration, whereas 180 min of two-legged dynamic knee-extensor exercise at ∼55% of their maximal workload (Steenberg et al. 2002) does not produce any change. From these studies it would appear that TNF-∝ secretion during exercise may be more dependent on the intensity of the exercise than its duration.

On the other hand, the change of cytokines reported in our study agreed with Petersen and Pedersen (2005) who suggested that IL-6 produced by contracting skeletal muscle is involved in glucose homeostasis during exercise, and inhibits TNF-∝ production. Suzuki et al. (2002) also suggested that IL-6 inhibits the release of pro-inflammatory cytokines IL-1β and TNF-∝ during exercise. The second explanation for the stable TNF-∝ concentration in our study is that an increasing IL-6 during the race inhibits further TNF-∝ secretion suppressing any additional increase between 100 and 200 km.

In summary, the results of our study demonstrate that changes in several biomarkers, indicative of muscle, cartilage, and liver damage, during a 200 km ultramarathon race were much more pronounced during the second half (100–200 km) of the race and that changes in plasma CPK are related to plasma LDH, Hs-CRP, ALT, AST and IL-6. These results suggest that ultra-endurance exercise is associated with a wide range of changes in injury-related parameters and that possibly greater damage occurs in the latter half of the race. We conclude that ultra-marathon running and other prolonged running events may be detrimental to muscle and cartilage structures increasing the incidence of degenerative conditions in later life.

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