

Changes in markers of muscle damage, inflammation and HSP70 after an Ironman triathlon race

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Abstract We investigated the effects of an Ironman triathlon race on markers of muscle damage, inflammation and heat shock protein 70 (HSP70). Nine well-trained male triathletes (mean \pm SD age 34 ± 5 years; $\dot{V}O_{2\text{peak}}$ $66.4 \text{ ml kg}^{-1} \text{ min}^{-1}$) participated in the 2004 Western Australia Ironman triathlon race (3.8 km swim, 180 km cycle, 42.2 km run). We assessed jump height, muscle strength and soreness, and collected venous blood samples 2 days before the race, within 30 min and 14–20 h after the race. Plasma samples were analysed for muscle proteins, acute phase proteins, cytokines, heat shock protein 70 (HSP70), and clinical biochemical variables related to dehydration, haemolysis, liver and renal functions. Muscular strength and jump height decreased significantly ($P < 0.05$) after the race, whereas muscle soreness and

the plasma concentrations of muscle proteins increased. The cytokines interleukin (IL)-1 receptor antagonist, IL-6 and IL-10, and HSP70 increased markedly after the race, while IL-12p40 and granulocyte colony-stimulating factor (G-CSF) were also elevated. IL-4, IL-1 β and tumour necrosis factor- α did not change significantly, despite elevated C-reactive protein and serum amyloid protein A on the day after the race. Plasma creatinine, uric acid and total bilirubin concentrations and γ -glutamyl transferase activity also changed after the race. In conclusion, despite evidence of muscle damage and an acute phase response after the race, the pro-inflammatory cytokine response was minimal and anti-inflammatory cytokines were induced. HSP70 is released into the circulation as a function of exercise duration.

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Introduction

The Ironman triathlon race consists of 3.8 km of swimming, 180 km of cycling and 42.2 km of running. Such ultraendurance exercise imposes strenuous physical load on the bodies of athletes, including significant energy demands (Kimber et al. 2002), thermal stress and dehydration (Sharwood et al. 2004), oxidative stress (Nieman et al. 2004) and muscle damage (Farber et al. 1991). Muscle damage after an Ironman triathlon probably results mainly from the marathon segment of the race (Hauswirth and Lehenaff 2001). Although several studies have reported changes in muscle

proteins in plasma as markers of muscle damage (Farber et al. 1991; van Rensburg et al. 1986), fewer data are available in relation to changes in muscle function and soreness after Ironman triathlons (Margaritis et al. 1999). Furthermore, until very recently, no studies had specifically examined the relationship between muscle damage and cytokine production following ultraendurance exercise (Nieman et al. 2005). Because of the shorter duration of the marathon segment in an Ironman triathlon, the relationship between muscle damage and cytokine production may differ from that following ultramarathon running events (Nieman et al. 2005).

Several studies have examined cytokine responses to endurance exercise such as ultramarathon running (Mastaloudis et al. 2004; Nieman et al. 2002, 2005), whereas there are fewer data available relating to cytokine changes after ultraendurance triathlon events (Jeukendrup et al. 2000; Nieman et al. 2004). The plasma concentrations of interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8 and IL-10 are significantly elevated following Ironman triathlon races (Nieman et al. 2004), whereas tumour necrosis factor (TNF)- α remains unchanged (Jeukendrup et al. 2000). No data are currently available relating to changes in other cytokines such as IL-1 β , IL-2, IL-4, IL-12p40, interferon (IFN)- γ and granulocyte colony-stimulating factor (G-CSF) after ultraendurance exercise. These cytokines play a key role in determining the balance of type-1 versus type-2 immune responses (Suzuki et al. 2002, 2003). The extreme demands of an Ironman triathlon race may alter the relative levels of these cytokines, and hence resistance to infection after the race.

There has been increasing interest in the role of heat shock proteins (HSPs) during exercise. The precise source and stimulus of the release of HSPs into the systemic circulation during exercise is currently unknown. There are reports of an increase in serum HSP72 concentration (also known as HSP70) after 2 h cycling at $\sim 65\%$ $\dot{V}O_{2\max}$ (Febbraio et al. 2004), 2 h treadmill running at 60% $\dot{V}O_{2\max}$, and after a marathon (Fehrenbach et al. 2005). Whether or not the additional physical demands of an Ironman triathlon cause greater changes in extracellular HSPs is currently unknown. Changes in extracellular HSPs have important implications for immunity, as these proteins are recognised as “chaperokines”, with key functions in mediating signal transduction and function within cells of the immune system (Asea 2005).

There were three aims to the present study. Firstly, we aimed to examine the changes in muscle function, soreness and plasma levels of muscle proteins to assess the level of muscle damage after an Ironman triathlon

race. Secondly, we aimed to investigate alterations in a broad range of pro-inflammatory, anti-inflammatory and immunomodulatory cytokines in plasma. Lastly, we aimed to assess changes in the plasma concentrations of HSP70 and other variables related to dehydration, haemolysis, liver and renal functions as indicators of the demands of the race and organ dysfunctions.

Materials and methods

Subjects

Nine well-trained male triathletes who completed the 2004 Ironman Western Australia triathlon participated in the study. The characteristics of the athletes are shown in Table 1. The athletes had all completed a minimum of one prior Ironman triathlon distance event in less than 10 h 30 min. Athletes read and signed an informed consent form conforming to the statement of protection for human subjects in the declaration of Helsinki, and the study obtained approval from the Institutional Human Research Ethics Committee. Athletes were allowed to eat and drink *ad libitum* during their race and no particular guidance was given to them as to what quantities or types of fluids and fuels they should consume.

Race conditions

The event consisted of a 3.8 km swim, followed by a 180 km cycle, and completed with a 42.2 km marathon run. Mean \pm SD environmental conditions ranged from 19–26°C ($23.3 \pm 1.9^\circ\text{C}$), and 44–87% relative humidity ($60 \pm 14\%$), with ocean temperature at 19.5°C. Performance times for the swim, cycle, and run phases, and the total time are shown in Table 1.

Table 1 Characteristics of subjects and their performance in the Ironman triathlon race

Age (years)	34.0 (5.0)
Height (m)	1.81 (0.58)
Weight (kg)	78.2 (5.5)
Sum of skinfolds (seven site) (mm)	49.5 (10.5)
$\dot{V}O_{2\text{peak}}$ ($\text{ml kg}^{-1} \text{min}^{-1}$)	66.4 (6.0)
Total race time	9:59:42 (0:34:38)
3.8 km swim time	0:56:43 (0:04:22)
180 km cycle time	5:11:52 (0:07:26)
42.2 km run time	3:51:05 (0:29:06)

Mean (SD) values of nine athletes are shown

Study design

Two days prior to the race, athletes reported to a makeshift laboratory at a rental home near the race site and a blood sample was taken from an antecubital vein. Muscle function tests and muscle soreness were also measured. Post race blood samples were taken within 30 min of athletes finishing the race, with the athlete lying supine in the medical tent, and between 14 and 20 h post-race at the makeshift laboratory. Muscle soreness was reassessed immediately and 14–20 h post-race, and muscle function tests were performed at 14–20 h post-race.

Criterion measures

Muscle strength

Maximal isometric strength of the knee extensors and knee flexors were measured using a strain gauge (Bongshin Loadcell Co. Ltd, Model DBBP 200, Korea) attached to a wire with a belt surrounding the ankle joint while athletes were seated on a specially designed chair to adjust the knee joint angle of 90°, and while athletes lay prone on a mat to adjust the knee joint angle of 90°, respectively. After two submaximal attempts, athletes were asked to generate maximal force for 3 s, and this measurement was taken twice with a rest of 60 s between attempts for both the knee extensors and the knee flexors. The intra-class correlation coefficient for the measurements of maximal isometric strength was 0.90. Peak value was displayed on a digital indicator (Rinstrum Pty. Ltd, Model 2100EX, Australia) connected to the strain gauge, and the higher value of the two measurements was used for further analysis. Grip strength was also measured using a Smedley grip dynamometer (Takei Scientific Instruments Co., Ltd, Model TKK 5001, Japan) for each arm twice, and the higher value was used for further analysis.

Vertical jump height

Vertical jump height was determined in two different types of jump; squat jump and counter movement jump using a leap meter (Takei Scientific Instrument, Japan). For the squat jump, athletes were asked to jump from the position holding the knee joint at 90° at least for 2 s without using arms that were placed on each side of the waist. The arm position was the same for the counter movement jump, but athletes started the jump motion from a standing position with counter movement. After two practice jumps, two trials for each jump were performed, and the higher value of the two

was used for further analysis. The intra-class correlation coefficients for the squat and counter movement jumps were 0.94 and 0.92, respectively.

Muscle soreness

Muscle soreness was assessed by a verbal rating scale of 0–10 with “no pain” for zero and “maximal (worst in my life)” for ten. Athletes were asked to rate their subjective soreness level on a questionnaire sheet while the investigator palpated muscles and they performed a movement that the investigator instructed such as squat and stretching. Muscle groups of the limbs and trunk such as the deltoid, pectoralis, biceps brachii/brachialis, triceps brachii, brachioradialis, rectus/obliquus abdominis, quadriceps femoris, biceps femoris, gastrocnemius/soleus, and low back muscles were assessed.

Blood markers

Approximately 7 ml of blood was drawn by a standard venipuncture technique from the antecubital vein using a vacutainer containing lithium heparin, and centrifuged for 10 min to obtain plasma. The plasma samples were frozen and stored at –20°C until the day of analysis.

Plasma cytokine concentrations were measured by two different methods. Initially, we attempted to measure the plasma concentrations of IL-2, IL-4, IL-6, IL-10, TNF- α and interferon (IFN)- γ using a Cytometric Bead Array (CBA) kit (BD Biosciences Pharmingen, San Diego, CA, USA). The CBA system is based on the use of fluorescent beads that provide a surface coated by antibodies for cytokine proteins. The beads are similar to the coated wells in a microplate of enzyme-linked immunosorbent assay (ELISA). The CBA assay offers a simultaneous detection of different cytokines using the same sample and greater dynamic range for measuring high cytokine concentration (Suzuki et al. 2003). However, IL-2, IL-4, TNF- α and IFN- γ were all below the detection limits of the assay in more than half of the plasma samples. Furthermore, we could not consistently detect IL-6 and IL-10 in all pre-exercise samples. Therefore, we chose to measure the plasma concentrations of IL-1 β , IL-1ra, IL-6 (Quantikine HS, R&D Systems, Minneapolis, MN, USA), IL-10, IL-12p40 (OptEIA, BD Biosciences), IL-4 (ANALYZA GT, Minneapolis, MN, USA) and G-CSF (EIA 96, IBL, Fujioka city, Japan) using ELISA. In addition to these cytokines, we measured the plasma HSP70 concentration (Stress Xpress, Stressgen Bioreagents, Victoria, BC, Canada) and myoglobin (Life Diagnostics, West Chester, PA, USA) by ELISA.

These concentrations were determined by comparison to a standard curve established in the same set of measurements. Measurements were made using a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA).

Plasma samples were also analysed for the following variables. Alanine aminotransferase (ALT, also known as glutamate pyruvate transaminase or GPT), aspartate aminotransferase (AST, also known as glutamate oxaloacetate transaminase or GOT), creatine kinase (CK), creatine kinase isoenzymes (CK-MM, CK-MB, CK-BB), aldolase, and lactate dehydrogenase (LDH) were measured as markers of muscle and liver damage. Serum amyloid A (SAA), C-reactive protein (CRP) and sialic acid were measured as indicators of the acute phase inflammatory response. Total protein, albumin, urea, uric acid, creatinine and calcium were measured to assess dehydration status and renal function. Lastly, bilirubin, alkaline phosphatase (ALP) and γ -glutamyl transferase (γ -GTP) were measured as markers of haemolysis and liver function, respectively. Total protein, albumin, urea, uric acid, creatinine, ALT, AST, CK, ALP, LDH, calcium and CRP were measured using an automated analyser (Model 747–400, Hitachi, Japan). Different analysers were used to measure free fatty acids, aldolase and sialic acid (Model 917, Hitachi, Japan), SAA and lipid peroxide (Model JCA-BM21, JOEL Ltd, Japan), and creatine kinase isoenzymes (K.K. Helena kenkyujyo, Saitama, Japan).

Statistical analysis

Data are presented as mean \pm SD. We checked the residuals for normal distribution, and applied a log transformation to the data for IL-1ra, IL-6, bilirubin and iron. We analyzed the data using a one-way repeated measure ANOVA. When we detected a significant main effect of time, we used paired *t*-tests and the false discovery rate procedure for multiple comparisons to determine specifically when differences occurred (Curran-Everett 2000). Statistical significance was set at $P < 0.05$. We used SigmaStat 3.1 (Systat Software, Point Richmond, CA, USA) for the data analysis.

Results

Muscle function

Maximal isometric strength of the knee extensors decreased significantly below pre-exercise values ($-0.3\times$

or 30%), but that of the knee flexors did not change significantly (Fig. 1). Squat jump and counter movement jump height decreased significantly, and the magnitude of decrease was significantly larger for squat jump ($-0.3\times$) compared with counter movement jump ($-0.2\times$). Grip strength did not change (pre: 48.6 ± 1.0 kg, 1 day post: 48.6 ± 1.5 kg).

Muscle soreness

Muscle soreness developed mainly in the lower limb muscles, and the knee extensors recorded their highest pain immediately and 1 day post-race, followed by ankle plantarflexors, and knee flexors (Table 2). Minor muscle soreness occurred to other muscles such as elbow extensors and flexors, deltoid and low back muscles, and abdominal muscles.

Cytokines

Soon after the race (within 30 min), the plasma concentrations of IL-1ra ($36\times$), IL-6 ($36\times$) and IL-10 ($53\times$) were all significantly elevated above pre-exercise values (Fig. 2a–c). Plasma IL-1ra ($0.6\times$) and IL-6 ($1.4\times$) concentrations remained significantly above pre-exercise values the day after the race. The race also significantly increased the plasma concentrations of G-CSF ($2.2\times$) and IL-12p40 ($0.3\times$) (Figs. 2d, 3a, respectively), however, only plasma G-CSF concentration ($1.9\times$) remained significantly elevated the day

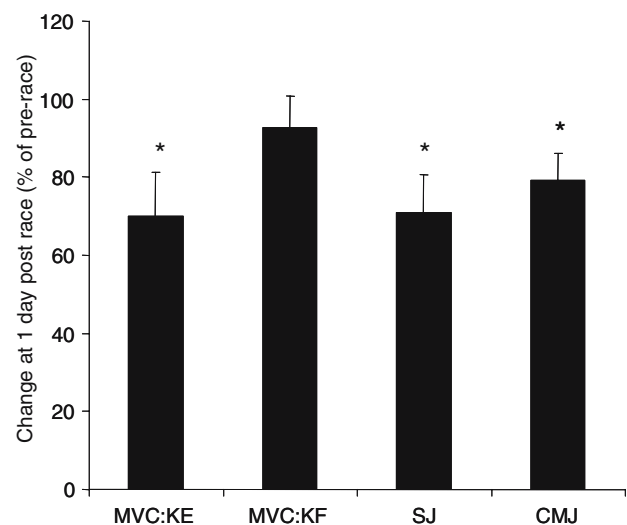


Fig. 1 Changes in maximal isometric strength of the knee extensors (*MVC:KE*) and the knee flexors (*MVC:KF*), squat jump height (*SJ*) and counter movement jump height (*CMJ*) at 1 day post-race relative to the pre-race value (100%). Data are mean \pm SD. *Significantly different from pre-race values $P < 0.05$

Table 2 Changes in muscle soreness in the knee extensors, knee flexors, and ankle plantarflexors before (Pre), immediately (Post) and 1 day after race (1 day post)

Muscle group	Pre	Post	1 day post
Knee extensors	0.6 (0.9)	6.1 (2.1)*	6.0 (2.7)*
Knee flexors	0.2 (0.3)	3.0 (1.8)*	3.4 (1.8)*
Ankle plantarflexors	0.1 (0.3)	4.3 (2.7)*	3.9 (3.0)*

Mean (SD) values of nine athletes are shown

* Significantly different from the Pre values $P < 0.05$

after the race. The changes in the plasma concentrations of IL-4 ($P = 0.163$) and IL-1 β ($P = 0.143$) were not statistically significant (Fig. 3b, c, respectively). As mentioned previously, we could not detect IL-2, TNF- α and IFN- γ in all plasma samples after exercise.

HSP70

Plasma HSP70 concentration was significantly elevated soon after the race (22 \times), whereas it had returned to pre-exercise values on the day after the race (Fig. 3d).

Markers of muscle damage and inflammation

Soon after the race, the plasma activities of CK (11 \times), aldolase (2.3 \times) and LDH (1 \times) were significantly elevated (Table 3). Plasma myoglobin concentration was also significantly elevated soon after the race (143 \times). One day after the race, the plasma activities of CK (28 \times) and aldolase (5.8 \times) were further elevated. In contrast, plasma LDH activity remained at a similar level, whereas plasma myoglobin concentration decreased but still remained above pre-exercise values (22 \times). The plasma activities of ALT (1.8 \times) and AST (7.5 \times) were significantly elevated on the day after exercise. CK derived from cardiac muscle (MB isoenzyme) also increased after exercise, but comprised less than 5% of the CK activity at all time points. Plasma CRP and SAA concentrations were not significantly elevated soon after the race, but they were significantly higher than pre-race values on the day after the race (31 \times , 48 \times , respectively). Sialic acid and lipid peroxide did not change significantly (data not shown).

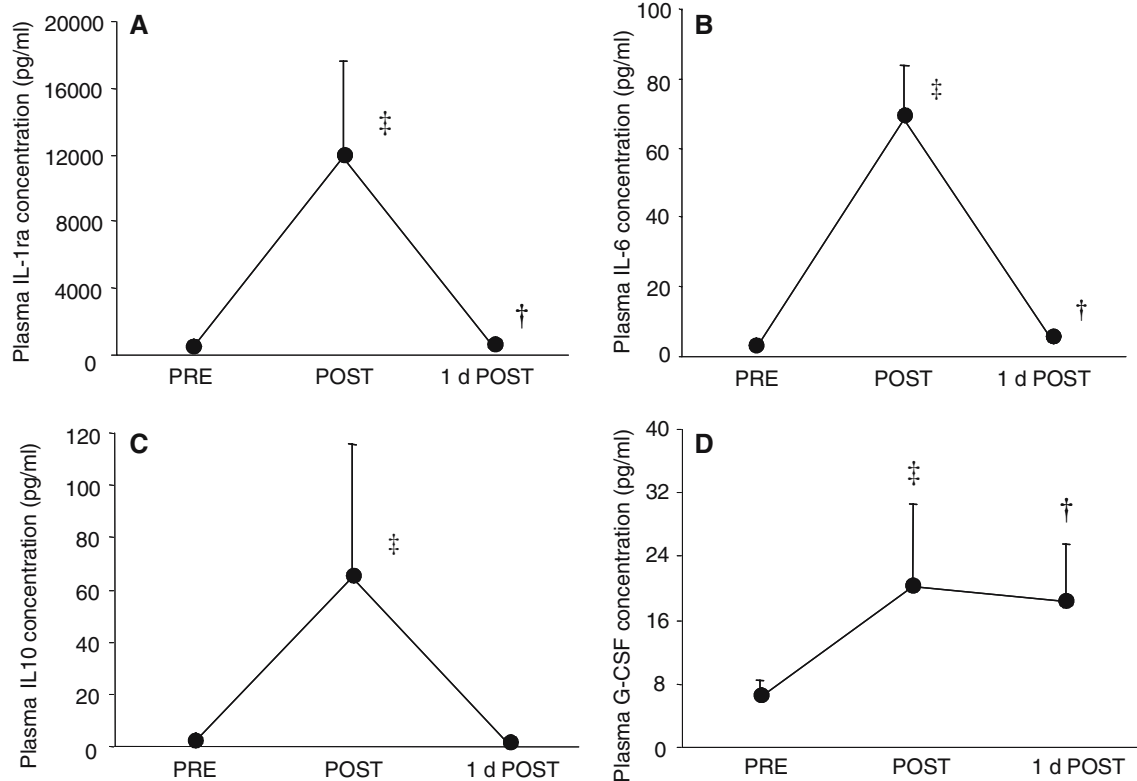


Fig. 2 The plasma concentrations of interleukin-1 receptor antagonist (IL-1ra) (a), IL-6 (b), IL-10 (c) granulocyte colony-stimulating factor (G-CSF) (d) before (PRE), immediately after (POST) and 1 day after (1 d POST) the race. Data are

mean \pm SD. ‡Significantly different from pre-exercise values $P < 0.001$; †significantly different from pre-exercise values $P < 0.01$

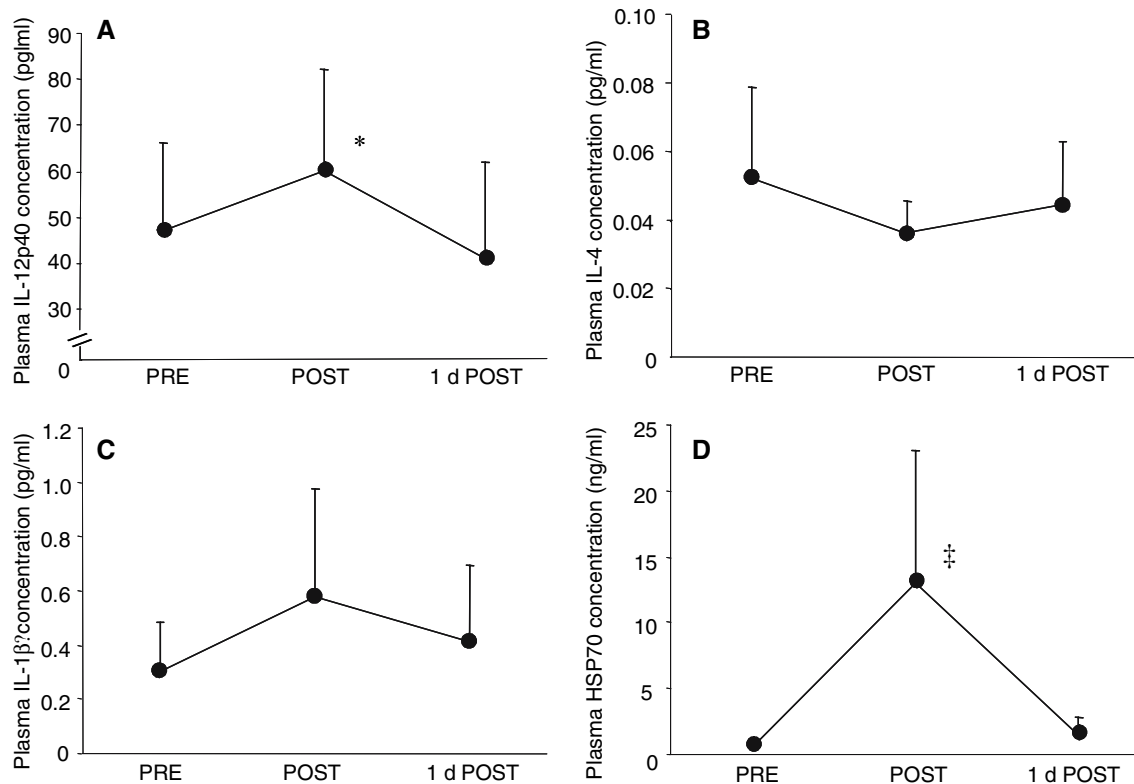


Fig. 3 The plasma concentrations of IL-12p40 (a), IL-4 (b), IL-1 β (c) and heat shock protein 70 (d) before (PRE), immediately after (POST) and 1 day after (1 d POST) the race. Data are

mean \pm SD. *Significantly different from pre-exercise values $P < 0.01$; ‡significantly different from pre-exercise values $P < 0.001$

Table 3 Changes in blood markers of muscle damage and inflammation before (Pre), immediately (Post) and 1 day after race (1 day post)

Variable (units)	Normal range	Pre	Post	1 day post	<i>P</i> value
CK (U/l)	50–230	210 (115)	2,436 (1,363)*	5,834 (3,075)‡	<0.001
CK-MM (U/l)	44–225	200 (111)	2,302 (1,137)*	5,606 (2,973)*	<0.01
CK-MB (U/l)	0–14	8 (3)	126 (105)*	227 (114)*	<0.05
ALD (U/l)	2.7–7.5	4.4 (1.2)	14.3 (6.6)*	29.8 (14.7)‡	<0.001
LDH (U/l)	120–245	180 (24)	285 (66)†	351 (111)‡	<0.001
ALT (U/l)	5–45	14 (4)	17 (6)	40 (17)‡	<0.001
AST (U/l)	10–40	29 (7)	93 (33)	249 (116)‡	<0.001
Mb (ng/ml)	12–90	21 (6)	2,998 (1,509)*	487 (249)‡	<0.001
CRP (mg/dl)	<0.45	0.10 (0.12)	0.24 (0.09)	3.15 (1.32)‡	<0.001
SAA (μ g/ml)	<8	4.8 (5.1)	23.0 (10.1)	230 \pm (101)‡	<0.001

Mean (SD) values of nine athletes are shown

*Significantly different from pre-exercise $P < 0.05$; †significantly different from pre-exercise $P < 0.01$; ‡significantly different from pre-exercise $P < 0.001$

CK creatine kinase, MM skeletal muscle-derived, MB heart-derived, ALD aldolase, LDH lactate dehydrogenase, ALT alanine aminotransferase, AST aspartate aminotransferase, Mb myoglobin, CRP C-reactive protein, SAA serum amyloid A

Clinical biochemical variables

Plasma total bilirubin concentration was significantly higher soon after the race (0.2 \times), and increased further above pre-exercise values (0.5 \times) on the day after the race (Table 4). Plasma creatinine (0.1 \times) and uric acid

(0.3 \times) concentrations were significantly elevated soon after the race, but both variables were similar to pre-exercise values the day after the race. In contrast, although urea also increased (0.2 \times), this was not statistically significant. Plasma total protein and albumin concentration did not change significantly during the

race, however, both variables decreased slightly (but significantly) below pre-exercise values on the day after the race. While plasma γ -GTP activity was not significantly altered soon after the race, it decreased below pre-exercise values ($-0.2\times$) on the day after the race. Plasma ALP activity was significantly lower than pre-exercise values soon after the race ($-0.2\times$). Plasma calcium concentration was significantly lower ($-0.1\times$) on the following day. Plasma free fatty acid concentration ($15\times$) increased significantly during the race, but returned to pre-exercise values on the day after the race.

Discussion

The main aim of this study was to investigate changes in markers of muscle damage and inflammation, cytokines and HSP70. Similar to previous studies examining long distance triathlons and other ultraendurance events, we observed decreased muscular function and increased muscle soreness after the race. These responses corresponded to increased plasma levels of muscle proteins and acute phase proteins. Despite this evidence of muscle damage and inflammation, the plasma concentrations of pro-inflammatory cytokines remained largely unchanged after the race. In contrast, IL-6 and the anti-inflammatory cytokines IL-1ra and IL-10 increased substantially. Therefore, after ultraendurance exercise such as an Ironman triathlon, the systemic pro-inflammatory response is limited. The large increase in plasma HSP70 concentration suggests that HSP70 is released systemically as a function of exercise duration. The alterations in the plasma concentrations of bilirubin, total protein, creatinine, albumin, urea and uric acid indicate that the race caused mild dehydration, haemolysis and possibly a minor decrease in renal blood flow.

Several studies have reported changes in markers of muscle damage after long distance triathlon races (Farber et al. 1991; Margaritis et al. 1999; van Rensburg et al. 1986). It is important to note that the time course of changes in muscular strength and muscle soreness does not necessarily correspond with changes in serum levels of muscle enzymes. It is commonly believed that neither muscle soreness nor the plasma/serum level of muscle enzymes accurately reflects the extent of muscle damage (Warren et al. 1999). Measures of muscle function such as strength and power may provide the most effective method for assessing the degree of muscle damage (Warren et al. 1999). In addition to the decrease in muscular strength, we also found that squat and countermovement jump height decreased after the race, and the decrease in squat jump height was greater than that of the countermovement jump, which is consistent with a previous study (Byrne and Eston 2002). Impaired jump performance may be due to factors such as reduced force and electromyographic activity, stretch-reflex sensitivity, muscle and joint stiffness (Byrne et al. 2004).

Plasma CK activity after the race was lower than that following ultramarathon running (Nieman et al. 2005; Rama et al. 1994), but was similar to that reported after other Ironman triathlons (Farber et al. 1991; van Rensburg et al. 1986). The plasma activities of aldolase, LDH, ALT and AST after the race were also similar to values after other Ironman triathlons (Farber et al. 1991; van Rensburg et al. 1986). The higher plasma CK activity following ultramarathon running cannot necessarily be attributed to greater muscle damage, but it might reflect the longer duration of running in such events. The finding that plasma CK, LDH, ALT and AST activities are altered in a consistent manner following Ironman triathlon events also suggests that exercise duration, in particular running duration, may influence changes in these variables.

Table 4 Plasma biochemical variables before (Pre), immediately after (Post) and 1 day after (1 day post) the race

Variable (units)	Normal range	Pre	Post	1 day post	<i>P</i> value
Creatinine (mg/dl)	0.65–1.09	0.96 (0.13)	1.10 (0.14) [‡]	0.94 (0.13)	<0.001
Uric acid (mg/dl)	<7	5.4 (0.8)	6.8 (1.0) [†]	5.5 (1.0)	<0.001
Urea (mg/dl)	8–20	20 (4)	25 (6)	22 (4)	0.116
Total protein (g/dl)	6.5–8.2	7.5 (0.2)	7.4 (0.5)	7.4 (0.4) [‡]	<0.001
Albumin (g/dl)	3.7–5.5	4.8 (0.3)	4.9 (0.4)	4.2 (0.3) [‡]	<0.001
Total bilirubin (mg/dl)	0.2–1	0.6 (0.3)	0.8 (0.3) [†]	0.9 (0.4) [‡]	<0.001
ALP (U/l)	104–338	232 (66)	225 (53) [†]	187 (52)	<0.01
γ -GTP (U/l)	16–73	23 (8)	24 (9)	19 (6) [‡]	<0.001
Calcium (mg/dl)	8.2–10	9.8 (0.3)	9.8 (0.6)	9.0 (0.5) [‡]	<0.001
Free fatty acids (mEq/l)	0.1–0.8	0.1 (0.1)	2.2 (0.5) [‡]	0.2 (0.1)	<0.001

Mean (SD) values for nine athletes are shown: γ -GTP, γ -glutamyl transpeptidase

[†]Significantly different from pre-exercise $P < 0.01$; [‡]significantly different from pre-exercise $P < 0.001$

Consistent with the responses to ultramarathon running (Mastaloudis et al. 2004; Nieman et al. 2002, 2005) and other Ironman triathlons (Nieman et al. 2004), the plasma concentrations of IL-1ra and IL-10 increased dramatically after the race. These responses were likely mediated by IL-6 (Steensberg et al. 2003), which also increased markedly in plasma after the race. In contrast, there were no significant changes in the plasma concentrations of the two major pro-inflammatory cytokines IL-1 β and TNF- α . This pattern of changes was consistent with a 42 km marathon race (Suzuki et al. 2000). We present new data indicating moderately elevated plasma concentrations of G-CSF and IL-12p40 after ultraendurance exercise. The extent of changes in these two cytokines was similar to a 42 km marathon race (Suzuki et al. 2003).

Endurance exercise increases the mRNA expression of TNF- α and IL-1 β within skeletal muscle, yet the systemic changes in these two pro-inflammatory cytokines are relatively minor (Nieman et al. 2003). Several possibilities exist to account for these findings. Firstly, TNF- α and IL-1 β may act locally rather than systemically (Petersen and Pedersen 2005). Secondly, the systemic release of TNF- α and IL-1 β may be restricted in response to exercise-induced muscle damage, or these cytokines may have a very short half-life in the systemic circulation. Lastly, stimuli other than muscle damage are required to elicit a strong systemic pro-inflammatory response. With respect to IL-6, changes in blood glucose availability and muscle glycogen depletion influence the mRNA expression and release of IL-6 from skeletal muscle into the systemic circulation during exercise (Febbraio et al. 2003; MacDonald et al. 2003; Nieman et al. 2003). In turn, changes in systemic IL-6 concentration during exercise most likely affect the synthesis of IL-1ra and IL-10 by other cells (Steensberg et al. 2003). Additional factors affecting plasma cytokine responses to exercise include epinephrine (Steensberg et al. 2001), hyperthermia (Rhind et al. 2004; Starkie et al. 2005), oxidative stress (Nieman et al. 2004), and possibly endotoxaemia (Camus et al. 1998; Jeukendrup et al. 2000).

Another aim of this study was to investigate changes in plasma HSP70 concentration after the race. Our data are consistent with previous findings that endurance exercise stimulates an increase in circulating HSP72 concentration (Febbraio et al. 2004). Febbraio et al. (2004) reported that carbohydrate ingestion during exercise attenuated the systemic release of HSP70 from hepatosplanchnic tissues, and suggested that this response may have occurred via a decrease in hepatic glucose production. Therefore, similar to the cytokine responses, the increase in plasma HSP70

concentration following the race in the present study may be related to increased metabolic demands and energy crisis. Extracellular HSP70 is believed to play an important role as a “chaperokine” that mediates signal transduction and function of immune cells (Asea 2005). However, further work is required to assess the biological significance of changes in extracellular HSP70 after exercise.

The final aim of this study was to compare changes in a variety of variables related to haemolysis, hydration and liver function with other forms of ultraendurance exercise similar in duration to the Ironman triathlon. The changes in plasma creatinine and uric acid concentrations were smaller than previously reported after a 100 km ultramarathon (Rama et al. 1994), a 230 km cycling event (Neumayr et al. 2003) and long-distance triathlons (Farber et al. 1991; van Rensburg et al. 1986). Unlike previous studies (Farber et al. 1991), plasma urea did not increase significantly after the race. The present observation that plasma total protein and albumin concentrations remained unchanged after exercise contrasts with previous data obtained after a 230 km cycling race (Neumayr et al. 2003) and another long-distance triathlon (Farber et al. 1991). However, the slight decline in these variables on the day after exercise is consistent with previous findings (Farber et al. 1991). These minor alterations likely reflect a decrease in renal blood flow, dehydration and/or plasma volume shifts following exercise.

The increase in plasma total bilirubin concentration was similar in magnitude to that reported previously after a long-distance triathlon (Farber et al. 1991), but smaller than the increase that occurred following a 100 km ultramarathon race (De Paz et al. 1995). Increased total bilirubin is partial evidence that haemolysis occurred during the race (De Paz et al. 1995). The shorter period of time spent running in the present study may account for the lower plasma total bilirubin concentration compared to a 100 km ultramarathon (De Paz et al. 1995).

In contrast to the findings of Rama et al. (1994), we observed a significant decrease in plasma γ -GTP activity 1 day after the race. γ -GTP is recognized as a sensitive, but non-specific marker of liver function. Values for plasma γ -GTP activity remained within the normal range after the race, and therefore the decline in the activity of this enzyme on the day after the race is probably not indicative of any change in liver function.

The change in plasma calcium concentration after the race in the present study contrasts with the responses to a 230 km cycling race (Neumayr et al. 2003). The reasons for this difference are unclear, but may be

related to the greater degree of muscle damage after the Ironman triathlon (Overgaard et al. 2004).

In summary, Ironman triathlon races cause substantial muscle damage and inflammation. There is a strong anti-inflammatory cytokine response to this form of ultraendurance exercise. Whether anti-inflammatory cytokines are produced mainly in response to muscle damage, or other factors following ultraendurance exercise, requires further investigation. Prolonged endurance exercise also stimulates an increase in plasma HSP70 concentration, but the source and precise stimulus for this increase is yet to be determined. Lastly, changes in clinical biochemical variables after ultraendurance exercise vary depending on the type and duration of exercise.

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