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ORIGINAL ARTICLE

Recovery after an Ironman triathlon: sustained inflammatory responses and muscular stress

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Abstract Ultra-endurance exercise, such as an Ironman triathlon, induces muscle damage and a systemic inflammatory response. As the resolution of recovery in these parameters is poorly documented, we investigated indices of muscle damage and systemic inflammation in response to an Ironman triathlon and monitored these parameters 19 days into recovery. Blood was sampled from 42 well-trained male triathletes 2 days before, immediately after, and 1, 5 and 19 days after an Ironman triathlon. Blood samples were analyzed for hematological profile, and plasma values of myeloperoxidase (MPO), polymorphonuclear (PMN) elastase, cortisol, testosterone, creatine kinase (CK) activity, myoglobin, interleukin (IL)-6, IL-10 and high-sensitive C-reactive protein (hs-CRP). Immediately post-race there were significant ($P < 0.001$) increases in total leukocyte counts, MPO, PMN elastase, cortisol, CK activity, myoglobin, IL-6, IL-10 and hs-CRP, while testosterone significantly ($P < 0.001$) decreased compared to prerace. With the exception of cortisol, which decreased below prerace values ($P < 0.001$), these alterations persisted 1 day post-race ($P < 0.001$; $P < 0.01$ for IL-10). Five days post-race CK activity, myoglobin, IL-6 and hs-CRP had decreased, but were still significantly ($P < 0.001$) elevated. Nineteen days post-race most parameters had returned to prerace values, except for MPO and PMN elastase, which had both significantly ($P < 0.001$) decreased below prerace concentrations, and myoglobin and hs-CRP, which were slightly, but significantly higher than prerace. Furthermore, significant relationships between leukocyte dynamics, cortisol, markers of muscle damage, cytokines and hs-CRP after the Ironman triathlon were noted. This study indicates that the pronounced initial systemic inflammatory response induced by an Ironman triathlon declines rapidly. However, a low-grade systemic inflammation persisted until at least 5 days post-race, possibly reflecting incomplete muscle recovery.

Keywords Ultra-endurance exercise · Muscle damage · Systemic inflammatory response · Immunoendocrine responses · Recovery phase

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Introduction

Regular physical training appears to enhance parts of the innate immune system while prolonged strenuous physical exercise attenuates many components of immunity (Gleeson 2007; Malm 2004; Pedersen and Hoffman-Goetz 2000). When the integrity of the organism is challenged by vigorous endurance exercise, a systemic inflammatory response is induced. This stereotypical and evolutionary conserved reaction to major physical stressors protects the organism by eliminating antigens, cellular debris, tissue fragments, by preventing further damage and by promoting tissue repair. However, it also elicits a temporary dysfunction of various aspects of immunity, which may increase the risk of subclinical and clinical infection (Fehrenbach and Schneider 2006; Gleeson 2007; Pedersen and Hoffman-Goetz 2000). The regulation of recruitment and distribution of leukocytes is complex and includes a plethora of signals and mediators such as hormones and cytokines (Malm

2004; Moldoveanu et al. 2001; Pedersen and Hoffman-Goetz 2000). Beyond the ultra-structural damage of muscle tissue as a potential stimuli for the production and release of cytokines, exhaustive endurance exercise induces several factors such as metabolic, hormonal, thermal and oxidative stress, all of which can give rise to the release of cytokines and other acute phase proteins in addition to the activation of several cell sub populations within the immune system (Fehrenbach and Schneider 2006; König et al. 2001). Nevertheless, crucially, up to now only very few studies have investigated the relationships between muscle damage and the release of cytokines or acute phase reactants explicitly after ultra-endurance exercise (Jeukendrup et al. 2000; Gomez-Merino et al. 2006; Nieman et al. 2004, 2005; Suzuki et al. 2006). Moreover, exercise-induced inflammatory or immune responses rarely have been followed longer than 1 day post-race (Gomez-Merino et al. 2006; Margaritis et al. 1997; Mastaloudis et al. 2004). Further questions arise on this topic as it is hypothesized that repetitive skeletal muscle tissue trauma caused by heavy training and competition could result in a persistent systemic cytokine response, which may be associated with a chronic inflammatory state, immune dysfunction and a poorly understood condition of overreaching/overtraining (Halson and Jeukendrup 2004) or underperformance syndrome (Robson-Ansley et al. 2007; Robson 2003; Smith 2000). Thus, detailed knowledge concerning the circumstances that alter the time-course of the restoration in muscular, inflammatory and immune parameters after ultra-endurance exercise is essential for designing training schedules that avoid eventual negative consequences of high volumes and intensities of physical activity.

Ironman triathletes are extraordinary in their level of training and in the endurance and intensity of exercise performed. Thus, they are an exceptional group to investigate not only because of their physiological characteristics and demands (Millet et al. 2003), the nutritional challenges (Jeukendrup et al. 2005), but urgently also to assess potentially harmful effects as a result of ultra-endurance sports in general (Knez et al. 2006). This is of particular importance since the number of non-professional athletes training for and competing in ultra-endurance events also continually increases. Furthermore, the clinical relevance of research work within this area is further emphasized because there are important similarities between the systemic inflammatory response to heavy endurance exercise and acute conditions such as sepsis and trauma (Fehrenbach and Schneider 2006; Pedersen and Hoffman-Goetz 2000).

The present study aimed to further elucidate the physiological, biochemical and molecular-biological stress responses to an Ironman triathlon in a large cohort of athletes. Specifically, we examined a range of muscular, inflammatory and hormonal parameters not only to quantify their magnitude directly after an acute bout of ultra-endurance exercise, but importantly also to verify the resolution of recovery. Information on the alterations in these responses *after* extremely demanding endurance exercise is limited. Therefore, it was a primary aim of the current study to get a thorough picture of these recovery responses including potential interactions between the immune system and skeletal muscle that are still not fully understood.

Materials and methods

Subjects

The study population comprised 48 non-professional well-trained healthy male triathletes, who participated in the 2006 Ironman Austria; 42 of them completed the study and were included in the statistical analysis. The characteristics of the subjects and their performance in the Ironman triathlon are shown in Table 1. The subjects were recruited from all over Austria half a year before the event. They were informed about the purpose and risks of the study before they provided written informed consent. The Ethics Committee of the Medical University of Vienna approved the study.

Table 1 Characteristics of the study participants and their performance in the Ironman triathlon

Age (y)	35.3 (7.0)
Height (cm)	180.6 (5.6)
Weight (kg)	75.1 (6.4)
BMI (kg m^{-2})	23.0 (1.2)
Body fat (%)	11.8 (4.1)
Cycling $VO_{2\ peak}$ (ml kg^{-1} min^{-1})	56.6 (6.2)
Power output at $VO_{2\ peak}$ (W)	358.3 (49.9)
Training over a period of 6 months prior to the Ironman triathlon	
Weekly net endurance exercise time (WNET) (h $week^{-1}$)	10.7 (2.6)
Swim training (km $week^{-1}$)	4.8 (2.2)
Cycle training (km $week^{-1}$)	144 (52.1)
Run training (km $week^{-1}$)	36.4 (10.6)
Performance in the Ironman triathlon	
Total race time (h:min:s)	10:51:52 (01:01:22)
3.8 km swim time (h:min:s)	01:09:51 (00:10:28)
180 km cycle time (h:min:s)	05:28:21 (00:29:08)
42.2 km run time (h:min:s)	04:08:26 (00:31:36)
Training after completion of the Ironman triathlon until 19 days post-race	
WNET (h $week^{-1}$)	4.2 (2.4)
Swim training (km $week^{-1}$)	2.1 (2.2)
Cycle training (km $week^{-1}$)	66.3 (53.8)
Run training (km $week^{-1}$)	12.3 (11.5)

Data are mean (SD), $n = 42$

Study design

All participants of the study were required to complete a medical and health-screening, a food frequency and a supplementation questionnaire and to document their training in the 6 months prior to the Ironman triathlon and thereafter until the end of the study (Table 1). All participants were not taking prescribed medication and avoided taking more than 100% of RDA in form of antioxidant supplements in addition to their normal dietary antioxidant consumption in the 6 weeks before the race and until the final blood sampling. Subjects were physically fit, free of acute or chronic illnesses, within a normal range of body mass index and non-smokers. In order to investigate exercise-induced effects in addition to recovery responses, blood samples were taken 2 days prerace, immediately (within 20 min), 1, 5 and 19 days post-race. Furthermore, subjects completed a 24 h dietary recall at each time point. The athletes abstained from intense exercise 48 h before the spiroergometry testing as well as before each blood sampling (except the Ironman itself). In addition, the athletes had fasted overnight before the 2-days prerace, 5- and 19-days post-race blood samples (which were all taken between 8:00 and 9:00 a.m.). On race day and 1 day post-race, they were allowed to drink and eat ad libitum, and the quantities of intake were recorded. After the triathlon the subjects performed "recovery" training that was of moderate intensity and duration until the end of the study (Table 1). During this recovery period the athletes were required to abstain from any training that was above the lactate threshold (respectively, the concomitant heart frequency) of each individual.

Race conditions

The Ironman triathlon was held in Klagenfurt, Austria on 16 July 2006 and consisted of 3.8 km swimming, 180 km cycling and 42.2 km running on a flat course. When the race started at 7:00 a.m. the air temperature and relative humidity were 15°C and 77%, with the lake temperature at 25°C. Between 4:00 p.m. and 5:00 p.m. respectively by finishing time (median time for subjects approximately 5:43 p.m.), air temperature reached a maximum and was 27.2°C, and relative humidity had decreased to 36% (data provided by the Carinthian Centre of the Austrian Central Institute for Meteorology and Geodynamics).

$VO_{2\ peak}$ testing protocol

The triathletes were tested 3 weeks before the race on a cycle ergometer (Sensormedics, Ergometrics 900). The maximal test protocol started at an initial intensity of 50 W, followed by 50 W increments every 3 min until exhaustion. During the test oxygen and carbon dioxide fractions (via Sensormedics 2900 Metabolic measurement cart), power output, heart rate, and ventilation were recorded continuously and earlobe blood samples for the measurement of the lactate concentration were taken at the beginning and at the end of each stage.

Blood sampling

At each blood sampling blood was collected into heparin, EDTA or serum vacutainers (Vacuette, Greiner, Austria). A field laboratory was installed at the race to ensure the appropriate collection of the first three blood samples. The blood was immediately cooled to 4°C and plasma separated at $1{,}711 \times g$ for 20 min at 4°C. Aliquots were immediately frozen at −80°C. Whole blood was taken for the hematological profile.

Hematological profile

The hematological profile was assessed with a MS4 Hematology 3-Part-Differential-Analyzer (Melet Schloesing Laboratories, Maria Enzersdorf, Austria). Exercise-induced changes in plasma volume were calculated (Dill and Costill 1974) until 5 days post-race to assess expansion of plasma volume, which persists for 3–5 days following the cessation of demanding exercise (Shaskey and Green 2000). All results are reported adjusted for these changes, except for cortisol and testosterone as for factors released in an endocrine manner it is important to consider their actual circulating concentration.

Plasma cortisol and testosterone concentrations

Both parameters were determined with radioimmunoassay based on the competition between radioactive and non-radioactive antigen. Testosterone was assessed with Active® Testosterone RIA DSL-4000 (Diagnostic Systems Laboratories, Inc., Webster, TX, USA), Cortisol with Corti-Cote® Cortisol Antibody Coated Tube - 125I RIA Kit (MP Biomedicals, Illkirch, France). The coefficients of variation were 5.3 and 6.4% for cortisol and testosterone, respectively.

Plasma myeloperoxidase (MPO) concentration and polymorphonuclear elastase (PMN) concentration

Myeloperoxidase concentrations were measured using the immundiagnostik MPO ELISA kit (Immundiagnostik AG, Bensheim, Germany) by two-site sandwich technique. The absorbance of samples and standards were read with a Fluostar Optima microplate reader (BMG labtechnologies, Germany) at 450 nm. Polymorphonuclear elastase was determined using a quantitative enzyme immunoassay (Milena Biotec GmbH, Bad Nauheim, Germany). All mea-

sures were made in duplicate. The coefficients of variation were 5.6 and 7.3% for PMN elastase and MPO, respectively.

Plasma markers of muscle damage and of inflammation

Plasma creatine kinase (CK) activity was detected using an automatic analyzer (Vitros DT 60 II module; Ortho-clinical Diagnostics, Germany). Concentrations of myoglobin and high-sensitive C-reactive protein (hs-CRP) were analyzed nephelometrically (Dade Behring, Marburg, Germany). Plasma interleukin (IL)-6 and IL-10 were determined by the Quantikine HS Immunoassay kit (R&D Systems GmbH, Wiesbaden, Germany). The coefficients of variation were 3.0, 4.5, 5.0, 7.8 and 8.6% for CK activity, myoglobin, hs-CRP, IL-6 and IL-10, respectively.

Data analysis

Data were tested for normal distribution using the Kolmogorov–Smirnov test. The main effect of time was obtained by using the repeated measures analysis of variance (ANOVA, general linear model). Dependent on normal distribution of data, either paired *t*-tests (for normally distributed data) or Wilcoxon tests (for not normally distributed data) were then used to assess differences in the test variables, whereas all post-race values were compared with prerace (= baseline) values. Either Pearson's (for normally distributed data) or Spearman's correlation (for not normally distributed data) was used to examine significant relationships. All statistical analyzes were performed using SPSS 15.0 for Windows. P values were considered as follows: $P < 0.05$ significant, $P < 0.01$ highly significant and $P < 0.001$ extremely significant.

Results

Race results

The completion time was 10 h 52 min ± 1 h 1 min (mean ± SD; Table 1). Three study participants failed to complete the race because of self-reported fatigue. In addition, three subjects could not participate in one or more blood sample time points and thus were excluded from the analysis.

Total leukocyte counts, leukocyte subpopulations and markers of neutrophil activation

Total leukocyte count increased significantly (+237%; $P < 0.001$) immediately after the Ironman triathlon versus prerace and remained significantly (+56%; $P < 0.001$) elevated until 1 day post-race. Changes in leukocyte subpopulations are shown in Fig. 1. Plasma MPO and plasma PMN

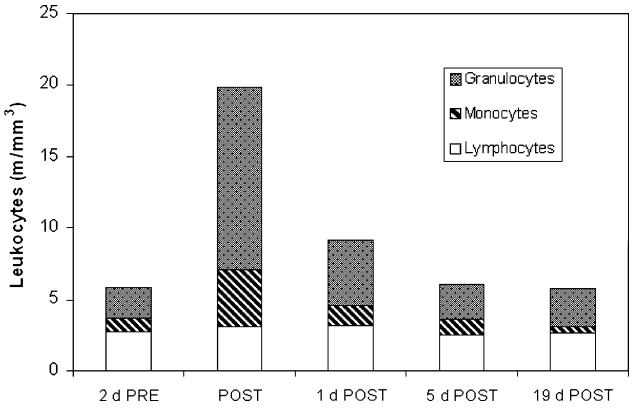

Fig. 1 Mean changes in blood concentrations of leukocytes and subpopulations 2 days prerace (*PRE*), immediately post-race (*POST*), 1 day post-race, 5 days post-race and 19 days post-race for 42 subjects

elastase concentrations significantly (both $P < 0.001$) increased by 342% and 424%, respectively immediately post-race. Both the variables remained elevated above the prerace values 1 day after the race (+70 and +108%, respectively; both $P < 0.001$). Myeloperoxidase, polymorphonuclear elastase values had returned to prerace 5 day post-race and 19 days post-race there was a significant (both $P < 0.001$) reduction (−28 and −21%, respectively) below prerace values (Table 2).

Plasma concentrations of cortisol and testosterone, and testosterone:cortisol ratio

There was a significant increase immediately post-race for cortisol (+241%; $P < 0.001$), whereas testosterone dropped below prerace (−53%; $P < 0.001$) at the same time point. One day post-race cortisol decreased sharply by 47% to below prerace values ($P < 0.001$). Five and nineteen days post-race cortisol concentrations remained moderately but not-significantly lower. The testosterone concentration levelled off below prerace values 1 day after the competition (−50%; $P < 0.001$) returning to prerace values 5 day post-race. The testosterone to cortisol ratio was significantly ($P < 0.001$) decreased by 86% directly after the race compared to prerace (Table 2).

Markers of muscle damage

Plasma CK activity increased significantly ($P < 0.001$) by 1,195% immediately post-race, with a maximum concentration observed 1 day post-race, when it was 4,316% higher than prerace values ($P < 0.001$). Creatine kinase values remained significantly ($P < 0.001$) higher than prerace until 5 days post-race (+281%). Plasma myoglobin peaked by 3,842% higher than prerace instantly post-race ($P < 0.001$), and remained significantly ($P < 0.001$) elevated by 964% higher than prerace values 1 day after

Table 2 Plasma values of myeloperoxidase (MPO), polymorphonuclear (PMN) elastase, cortisol, testosterone and testosterone:cortisol ratio

	Pre	Post	1 day post	5 days post	19 days post	Time effect (*P*)
MPO (μg L^{-1})	57 (31)	253 (122)***	97 (82)***	61 (58)	41 (25)***	<0.001
PMN elastase (μg L^{-1})	46 (23)	239 (137)***	95 (104)***	44 (31)	36 (16)***	<0.001
Cortisol (nmol L^{-1})	282 (112)	957 (696)***	149 (66)***	249 (107)	273 (110)	<0.001
Testosterone (nmol L^{-1})	11.4 (5.6)	5.3 (3.6)***	5.5 (2.9)***	12.7 (6.9)	12.3 (6.3)	<0.001
Testosterone:cortisol ratio	0.040 (0.037)	0.006 (0.009)***	0.037 (0.027)	0.051 (0.036)	0.045 (0.032)	<0.001

Values are mean (SD); $n = 42$

Pre 2 days prerace; *Post* immediately post-race; *1 day post* 1 day post-race; *5 days post* 5 days post-race; *19 days post* 19 days post-race

*** Significantly different from prerace values, $P < 0.001$

the race. Thereafter myoglobin concentrations declined, but remained elevated by 5 days (+45%) and 19 days (+30%) after the competition (both $P < 0.001$) (Fig. 2).

Plasma cytokines and high-sensitive C-reactive protein concentrations

Plasma IL-6 increased dramatically in response to the race (+10,408%; $P < 0.001$), and despite a sharp decline, values remained significantly elevated 1 day (+345%; $P < 0.001$) and 5 days (+79%; $P < 0.001$) after the race. The plasma IL-10 concentration was also elevated immediately after the race (+287%; $P < 0.001$), and remained above prerace concentrations 1 day post-race (+37%; $P < 0.01$). Five days post-race, IL-10 had declined by 4% below prerace concentrations ($P < 0.05$), and 19 days post-race levels were similar to prerace. The plasma hs-CRP concentration rose significantly ($P < 0.001$) immediately after the race by 543% and had increased by 7,702% 1 day post-race ($P < 0.001$). High-sensitive C-reactive protein subsequently decreased, but values remained significantly higher than prerace concentrations 5 days (+881%; $P < 0.001$) and 19 days after the competition (+38%; $P < 0.01$) (Fig. 3).

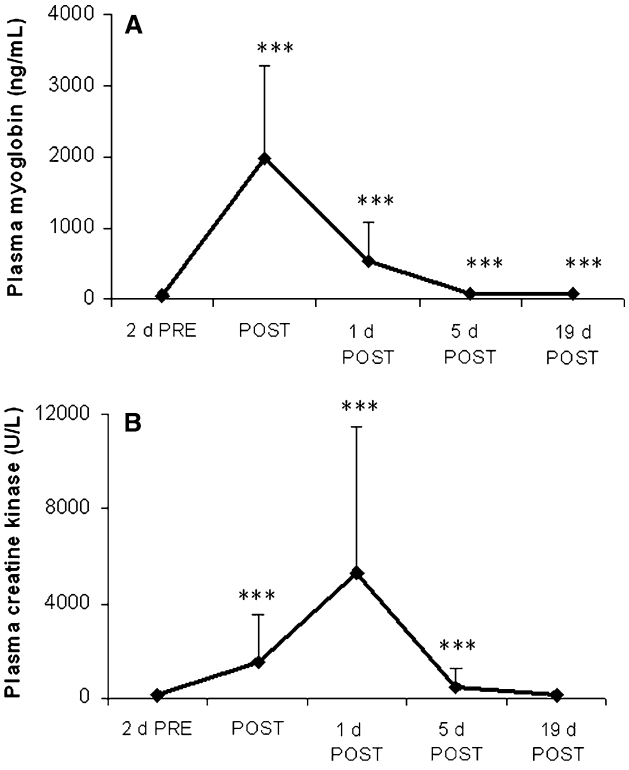

Fig. 2 Changes in plasma myoglobin concentrations (**a**) and plasma creatine kinase activity (**b**) 2 days prerace, immediately post-race, 1 day post-race, 5 days post-race and 19 days post-race. Data are mean ± SD; $n = 42$; ** significantly different from prerace values, $P < 0.001$

Associations with neutrophil dynamics, changes in cytokines and hs-CRP

Significant positive correlations were obtained between the change in total leukocyte count and changes in plasma myoglobin, CK activity, cortisol and IL-6, which are summarized in Table 3. Moreover, a positive correlation was observed between the pre- to immediately post-race change of IL-6 and the pre- to 1 day post-race change of MPO ($r = 0.45$; $P < 0.01$). Moderate positive correlations were observed between the pre- and post-race changes of IL-6 and markers of muscle damage. In addition, the pre- to immediately post-race responses in IL-10 correlated positively with cortisol ($r = 0.46$; $P < 0.01$) and were inversely related with performance-associated variables (Table 3). There were also several stronger positive correlations between hs-CRP and markers of muscle damage that are also shown in Table 3.

Discussion

The time-course of recovery after acute ultra-endurance exercise is a critical question that has rarely ever been answered. By quantifying the recovery responses until 19 days after the race in a large cohort of Ironman competitors we addressed this issue in a comprehensive manner.

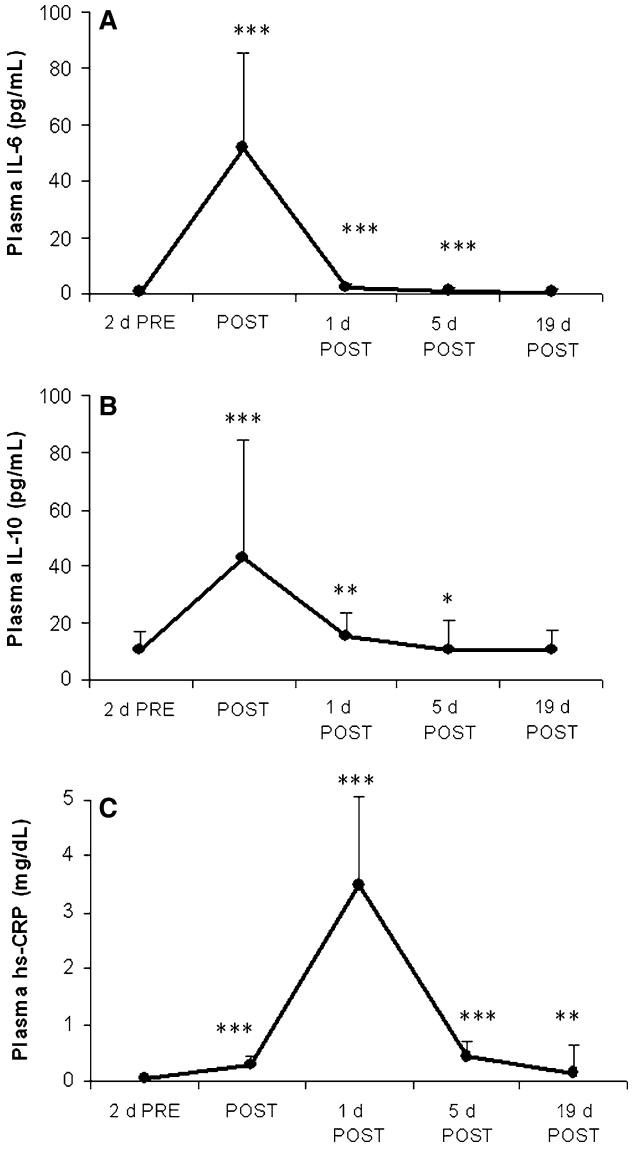

Fig. 3 Changes in the plasma concentrations of interleukin (IL)-6 (**a**), IL-10 (**b**) and high-sensitive C-reactive protein (hs-CRP) (**c**) 2 days prerace, immediately post-race, 1 day post-race, 5 days post-race and 19 days post-race. Data are mean ± SD; $n = 42$; *** significantly different from prerace values, $P < 0.001$; ** significantly different from prerace values, $P < 0.01$

The main finding of the present study is that markers of muscle damage, IL-6 and hs-CRP were still altered 5 days after the long-distance triathlon. Moreover, the large number of study participants enabled us to substantiate scarce findings regarding associations between muscular stress and the inflammatory response with a high statistical power.

The response of the steroid hormones following the Ironman triathlon revealed the major systemic stress caused by the competition. Corresponding to previous studies (Bishop et al. 2003; Davison and Gleeson 2006; Suzuki et al. 2000), plasma cortisol rose significantly, whereas testosterone decreased in response to prolonged exercise. While plasma testosterone remained lower than prerace 1 day post-race, cortisol levels recovered and, probably also reflecting the circidian rhythm of steroidhormones, fell below prerace values. The ratio of testosterone to cortisol that hypothetically indicates the balance between anabolic and catabolic effects of exercise (Halson and Jeukendrup 2004) declined as an immediate response to the triathlon, but normalized within the first day of recovery. Exercise-induced changes of both hormones are known to affect the immune system (Pedersen and Hoffman-Goetz 2000), and in the present study cortisol was associated with the exercise-induced leukocytosis as well as with the increase of IL-10.

In agreement with other studies examining muscle damage following a long distance triathlon (Gomez-Merino et al. 2006; Margaritis et al. 1997; Suzuki et al. 2006), the initiate injury of skeletal muscle injury was indicated by the leakage of myofibre proteins into the blood plasma. Mechanical stress (associated with exercise involving frequent eccentric or lengthening contractions such as in the marathon split of the Ironman triathlon) in addition to metabolic stress are believed to be the most important initial factors leading to exercise-induced muscle damage (Tee et al. 2007). In the present investigation, the time-course of muscle damage markers is similar to that characteristically found after protocols, in which metabolic stress is suggested to be heavily involved in muscle injury (Margaritis et al. 1997; Tee et al. 2007; Whyte et al. 2000). The highest circulating myoglobin concentrations were found immediately after the race and, presumed that there was no further increase according to previous data (Whyte et al. 2000), CK activity also peaked within 1 day after the triathlon. Since the training intensity of the subjects in the first day after the Ironman race was only modest compared to the tapering period before the competition, the prolonged appearance of myofibre proteins in the plasma until at least 5 days post-race is most likely related to subsequent muscle repair processes and probable local inflammatory responses. Although data is not consistent (St Pierre Schneider and Tiidus 2007), there is good evidence to suggest that neutrophils and macrophages infiltrate damaged muscle (St Pierre Schneider and Tiidus 2007; Tidball 2005). Even though this is a desirable response in terms of muscle repair and probably also muscle adaptation (Malm 2004; Tidball 2005) it may trigger further muscular inflammatory processes and damage, partly through the increased formation of reactive oxygen species (König et al. 2001). The slightly, but significantly elevated plasma myoglobin concentration 19 days post-race might rather be attributed to the gradual resumption of run training (despite its moderate intensity and volume) than to direct race responses. Otherwise, this could also indicate that muscle regeneration was not complete (Tee et al. 2007).

Table 3 Significant associations with exercise-induced responses of total leukocyte counts, interleukin (IL)-6, IL-10 and high-sensitive C-reactive protein (hs-CRP)

	MPO	Cortisol	CK	Myoglobin	IL-6	Total race time	Run split
Leukocytes							
Δ pre to 1 day post		Δ pre to post: 0.42**	Δ pre to 1 day post: 0.47**	Δ pre to post: 0.44**; Δ pre to 1 day post: 0.44**	Δ pre to 1 day post: 0.48**		
IL-6							
Δ pre to post	Δ pre to 1 day post: 0.45**		Δ pre to 1 day post: 0.35*	Δ pre to 1 day post: 0.38**			
IL-10							
Post						−0.43**	−0.47**
Δ pre to post		Δ pre to post: 0.44**					−0.42**
Hs-CRP							
Post			post: 0.44**				
1 day post			1 day post: 0.52***	1 day post: 0.58***			
Δ pre to 1 day post			Δ pre to post: 0.60***; Δ pre to 1 day post: 0.54***	Δ pre to post: 0.61***			
5 days post			5 days post: 0.53***				

MPO myeloperoxidase; *CK* creatinkinase; *Post* immediately post-race; *1, 5, 19 post* 1, 5, 19 days post-race; *Δ pre to post* change from pre- to immediately post-race; *Δ pre to 1 day post* change from pre- to 1 day post-race

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

With regards to the cellular immune system the systemic inflammatory response was characterized by a pronounced leukocytosis immediately after the Ironman triathlon (Fig. 1). Subsequently, the total leukocyte count declined, but still significantly increased until 1 day post-race. Despite a significant decrease in lymphocyte percentages, there was an increase in total lymphocyte counts post- and 1 day post-race, whereas lymphocyte concentrations tend to decrease 5 days after the competition. In support of previous studies (Peake et al. 2005b; Suzuki et al. 1999), a number of correlations between CK and myoglobin changes and alterations in total leukocyte count within the present study indicate that polymorphonuclear leukocytes (i.e., primarily neutrophils) are mobilized in response to exercise-induced muscle damage. The increase in circulating neutrophils following muscle damage is possibly due to activation of the alternative complement pathway and stimulated by the appearance of damaged muscle tissue fragments in the blood (Peake et al. 2005b; Suzuki et al. 1999). Inflammatory mediators such as IL-6 have also been associated with the release of leukocytes [i.e., particularly neutrophils (Suzuki et al. 2003)], which is supported by the present data. Furthermore, the observed associations between the exercise-induced increase in cortisol and the changed leukocyte numbers 1 day after the competition provides further evidence to the proposed time-lapsed role of cortisol in leukocyte trafficking (König et al. 2001; Malm 2004; Pedersen and Hoffman-Goetz 2000). In addition, the amplified response of plasma concentrations of MPO and PMN elastase in the course of the Ironman triathlon reflects a rapid neutrophil activation and degranulation. Despite a marked decrease towards prerace, both granular enzymes remained elevated after 1 day of recovery. In the case of MPO, the observed relationship between its change in plasma from pre- to 1 day post-race and the the IL-6 response immediately after the race may point to a delayed IL-6 stimulated neutrophil degranulation, as shown in a study of marathon running (Suzuki et al. 2003). Only few studies have investigated MPO levels following a marathon race (Melanson et al. 2006; Suzuki et al. 2003) and the present study appears to be the first to investigate the effects of an Ironman triathlon or exercise of a similar duration on MPO. Myeloperoxidase is also used as a novel marker for myocardial injury (Melanson et al. 2006). However, data within the current study indicate that elevations in MPO as well as in PMN elastase most likely were part of the exercise-induced systemic inflammatory response, as the post-race increase in cardiac troponin T and brain natriuretic peptide were not associated with inflammatory stress (König et al. 2007). Both enzymes possess lytic capacities and thus they assist in the destruction of damaged tissue or in the destruction of infectious agents (Bishop et al. 2003; Peake et al. 2005b; Tidball 2005).

IL-6 is one of the most potent mediators of the early exercise-induced systemic inflammatory response and, consistent with the findings of previous ultra-endurance studies (Jeukendrup et al. 2000; Mastaloudis et al. 2004; Nieman et al. 2002, 2004; Suzuki et al. 2006), plasma IL-6 concentration rose considerably (greater than 100-fold) following the race. The relationship between the leukocytosis and the rise in IL-6 1 day after the competition probably indicates that IL-6 has promoted leukocyte mobilization. A similar relationship is also reported in a previous marathon study (Suzuki et al. 2003). In addition, we found correlations between 1 day post-race changes of IL-6 and myoglobin along with CK, but these associations were weaker than directly after a 160-km run race (Nieman et al. 2005). It is plausible, that, lined up with recent research on that topic (Fischer 2006; Pedersen et al. 2007), contracting muscles account for the release of IL-6. Nevertheless, there is evidence to suggest that exercise-induced muscle damage is not solely responsible for the simultaneous initial increase of plasma IL-6 (Peake et al. 2005b) and that other signals such as low glycogen stores may play a more important role (Pedersen et al. 2007). Previous studies have reported that plasma IL-6 concentrations had returned to prerace values 1 day post-race after an ultramarathon (Mastaloudis et al. 2004) and after an Ironman triathlon (Jeukendrup et al. 2000), whereas after another Ironman race IL-6 levels were significantly elevated 1 day post-race (Suzuki et al. 2006), but IL-6 concentrations were not documented more than 1 day post-race. Importantly, we found that IL-6 levels fell 1 day after the long-distance triathlon, but were sustained significantly above prerace values until 5 days post-race. Similarly, Robson-Ansley et al. (2007) reported a low-grade, but prolonged elevation of systemic IL-6 following an acute period of intense running training. The migration of cytokine-releasing macrophages involved in muscle repair and/or in persistent glycogen depletion is suggested to be the main reasons for the long-lasting post-exercise elevation in IL-6 (Robson-Ansley et al. 2007). Even despite an adequate carbohydrate intake, exercise-induced muscle damage can impair appropriate glycogen re-synthesis (Jeukendrup et al. 2005) and it is known that carbohydrate availability in skeletal muscles modulates IL-6 production (Pedersen et al. 2007). Thus, the low-level, but long-standing IL-6 response into recovery in the present study may be associated with a delayed glycogen restoration as well as with regenerative processes modulated by recruited inflammatory cells (as another potential source of IL-6 production).

Consistent with previous studies (Nieman et al. 2002; Suzuki et al. 2003, 2006), we found increased IL-10 concentrations immediately after the competition and IL-10 values remained significantly elevated until 1 day after the race. Based on the concept that the appearance of counter-regulatory anti-inflammatory cytokines such as IL-10 after strenuous exercise attenuates the inflammatory and immune response to prevent overshooting inflammation, it is suggested that well-trained athletes are able to balance the acute exercise-induced inflammation (Fehrenbach and Schneider 2006). Results of a recent study in Ironman triathletes imply that there is a strong compensatory anti-inflammatory cytokine response (Suzuki et al. 2006). Our data support this idea because the pronounced *initial* inflammatory response was rapidly diminished in the course of the recovery from the competition. Regarding the potential stimuli of the anti-inflammatory cytokine response, the present data provide further indirect evidence that exercise intensity is a major factor in the elevation of IL-10. In a study investigating well-trained male runners, Peake et al. (2005a) reported that factors related to the intensity of exercise such as stress hormones had a stronger influence on the production of anti-inflammatory cytokines than muscle damage. In line with these results, we observed that the exercise-induced change in IL-10 was negatively related to the run split time as well as the change in cortisol immediately post-race. Furthermore, the IL-10 increase was negatively correlated with the total race time and the run split time (i.e., IL-10 concentration rose with performance).

The delayed increase of CRP following intense endurance exercise is a sign that the inflammatory response had reached a systemic level (Pedersen and Hoffman-Goetz 2000). C-reactive protein is released from the liver induced by IL-6 (Fischer 2006) and is responsible for the recognition and clearance of damaged cells (Plaisance and Grandjean 2006). C-reactive protein has also been established as a novel marker of inflammation and is suggested to provide additional information concerning atherosclerotic lesions (Plaisance and Grandjean 2006). Whereas evidence increases, that regular physical activity reduces CRP concentrations (Plaisance and Grandjean 2006), information concerning the response of CRP to an acute bout of ultra-endurance exercise is limited (Kim et al. 2007; Mastaloudis et al. 2004; Suzuki et al. 2006). In the present study, plasma CRP concentrations were significantly increased even immediately post-race, which is in contrast to another study on Ironman finishers (Suzuki et al. 2006). This rapid response in plasma CRP was followed by a marked further elevation 1 day post-race. Thereafter levels declined, but were still augmented 5 days post-race corresponding to the results of another ultra-endurance study, in which CRP levels after an ultra-marathon were determined over a longer time-course (Mastaloudis et al. 2004). Crucially, we found a number of associations between CRP and markers of muscle damage within 20 min, 1 day and 5 days after the Ironman. Therefore, our data support very recent findings (Kim et al.

2007) that muscle damage and the subsequent repair processes are important inducers in CRP. The slightly elevated CRP levels 19 days post-race (in parallel with moderately increased myoglobin concentrations) might be related to incomplete muscle recovery, but alternatively, this could also be a sign of low prerace values.

Interestingly, the three subjects, who dropped out from the race due to self-reported fatigue symptoms (but without any apparent systemic symptoms of an infection) had much higher prerace CRP concentrations than any other study participant with levels ranging from 316 to 1,442% above the mean prerace concentration. In two of them, these values exceeded the normal clinical range, and in parallel noticeable higher MPO levels (in both) and IL-6 concentration that was nearly 300% higher than the mean prerace IL-6 level (in one subject) were observed. The anomalies in these inflammatory markers in these subjects may represent a prolonged inflammatory condition following the prolonged training period and the subsequent tapering and thus might provide some additional evidence to the cytokine hypothesis of the unexplained underperformance syndrome (Robson-Ansley et al. 2007; Robson 2003; Smith 2000).

The most important finding of the current study was that although the marked initial inflammatory response induced by Ironman triathlon subsided rapidly, a low-grade systemic inflammatory response was sustained for at least 5 days of recovery. The prolonged moderate, but significant elevation of IL-6 and CRP might be associated with inflammatory processes and/or impaired glycogen replenishment within damaged muscle. Athletes may be more susceptible to infections due to this attenuated immune competence within this first period of recovery after demanding endurance exercise. Furthermore, inadequate rest following prolonged, intensive exercise might cause a chronic systemic inflammatory state that in turn leads to a syndrome of impaired performance and progressive fatigue. However, due to the continuous demands of Ironman competition on training schedules, competitive athletes might not have sufficient recovery between the races. Thus, finding an appropriate balance between training, competition and recovery is an essential challenge to maintain a high level of performance and to minimize potential health consequences. From the perspective of the observed muscle repair and inflammatory processes at least 2–3 weeks of active recovery is advisable before gradually returning to more intensive training.

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