Comparison of systemic cytokine responses after a long distance triathlon and a 100-km run: relationship to metabolic and inflammatory processes

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ABSTRACT. Suggested mechanisms for the systemic, circulating cytokinemia observed during heavy physical exertion include inflammation and energy demand. We compared cytokine levels and examined the underlying physiological mechanisms between a long-distance triathlon and a 100-km run, two endurance races of similar duration but characterized by differences in muscle strain. Blood samples were collected from 12 triathletes (34.8 ± 1.4 yr) and 11 runners (42.4 ± 2.2 yr) the day before and at the end of races (T1, R1), and 24 h and 7 days post-race (R2, R3). At R1, significant race-related differences were observed, with greater increases in plasma levels of interleukins (IL)-6, IL-1ra, and IL-10 in the triathletes than in the runners, while levels of the chemokine IL-8 increased solely in the runners (P < 0.05, P < 0.05, P < 0.01, and P < 0.001, respectively). At R1, free fatty acid (FFA) levels were 119% higher in the triathletes than in the runners, who were the most liable to muscle damage in view of increased levels of the muscle-specific enzyme, creatine kinase (CK), loss of muscle flexibility and decreased physical performance. At R1, levels of heat shock protein (HSP)72 increased in the two groups but were 173% higher in the runners. For the two groups, all parameters had returned to pre-race levels by seven days post-race. Positive correlations were noted between IL-6 and FFA in the triathletes and between IL-8 and CK and HSP72 in the runners. The differences between cytokine responses after a long distance triathlon and a 100-km run suggested that IL-6 and IL-8 could be employed as respective markers of the intensity of the muscular activity required for substrate availability and vascular inflammation.

Keywords: cytokines, metabolic and inflammatory processes, long-distance triathlon, 100-km run
The water temperature was 18.6°C at the start. (7.00 hours), with a high of 24.1°C around 14.00 hours. Swimming in open water, 120 km cycling with a total uphill grade of 1000 m, and 30 km running (on a coastal road).

The LD Triathlon held in September comprised 4.0 km swimming, 120 km cycling, and 30 km running due to the fewer eccentric contractions with a consequent difference in cytokine production. This was indicated by results of a previous study showing higher levels of muscle-specific enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) in the blood of athletes at the end of and 4 days after a 100-km race compared to a long-distance triathlon (the Nice long-distance triathlon), suggesting that there was more muscle damage in the endurance running race [20]. We also investigated relationships between cytokine changes and the demand for substrate availability, muscle injuries (soreness and biochemical markers of damage), hormonal inflammatory responses, and inducttion of the extracellular, soluble heat shock protein 72 (HSP72) in the blood.

**Materials and Methods**

**Subjects and race description**

The study was approved by a French medical ethics committee (Faculty of Medicine, Paris V, France). After signing an informed consent form, 12 male, trained, long-distance triathletes (mean age 34.8 ± 1.4 years, VO_2max 71.3 ± 1.8 mL/kg/min) and 12 male, trained, ultramarathoners (mean age 42.4 ± 2.2 years, VO_2max 69.3 ± 1.1 mL/kg/min) participated in the International Long-distance Triathlon in Nice (France), and in the 100-km run in the Loire region of France. These long-distance competitions were chosen as they were of comparable duration. The LD Triathlon held in September comprised 4.0 km swimming in open water, 120 km cycling with a total uphill of 1000 m, and 30 km running (on a coastal road). The temperature was 13.5°C in the morning at the start (7.00 hours), with a high of 24.1°C around 14.00 hours. The water temperature was 18.6°C at the start.

The 100-km Run held in June was performed on country roads. The race started and finished at the same altitude, with a total uphill grade of 1000 m. The temperature was 9.5°C in the morning at the start (5.00 hours), with a high of 25.5°C around 14.00 hours.

Subjects consumed food and beverages *ad libitum* during the races.

**Testing procedures**

Clinical examination took place on four sessions: the day before the race (T1), on the race day at the end of the race (R1), 24 h and 7 days post-race (R2, R3). To account for the effects of circadian rhythms, all tests were conducted at the time when subjects would be completing the race: between 12 am and 5 pm for the 100-km Run and between 2 and 5 pm for the LD Triathlon. General health was assessed, with emphasis on muscle/joint-based problems. The physician measured muscle flexibility (quadriceps), with the subject lying face down, as the distance in cm to retractor the leg toward the thigh: the greater the distance, the lower the flexibility. In addition, subjects were asked to rate their muscle and joint soreness on a ratio scale (Likert scale of muscle soreness) of zero to six, where zero is the complete absence of problems and six the most severe [21]. The subjects were weighed in all sessions. On the fourth session, subjects performed vertical jump tests for assessment of lower body explosive power (counter movement jump with 90° knee flexion before the extension) [22]. In the 100-km running race, three subjects were unable to complete the 100 km, one, who only ran 33 km, was excluded from the study, but the two others were tested as they had run 80 km.

**Blood sampling and analysis**

Approximately 20 mL of blood were taken from an antecubital vein using sterile, venipuncture techniques after the subjects had rested supine for 10 minutes under quiet conditions. Serum or plasma were stored at -80°C in our laboratory and used for subsequent determinations. Plasma IL-6, IL-8, IL-10, IL-1ra, tumor necrosis factor alpha (TNF-α) and heat shock protein (HSP)72 concentrations were determined in duplicate using sensitive and highly sensitive sandwich ELISA kits, provided by R&D Systems, Biosource and Stressgen (USA and Canada). The minimum detectable concentrations were < 0.039 pg/mL for IL-6, < 0.1 pg/mL for IL-8, < 0.5 pg/mL for IL-10, < 4 pg/mL for IL-1ra, < 0.09 pg/mL for TNF-α, and 0.2 ng/mL for HSP72. In serum, CRP (C-reactive protein) concentrations were assayed using a sensitive, turbidimetric method on an automatic analyzer (Olympus AU600 Application; Olympus France) with a sensitivity limit of 0.8 mg/L. In plasma, CK, free fatty acids (FFA) and glucose concentrations were measured using commercial colorimetric methods (Biomérieux, Boehringer Mannheim, France). Cardiac troponin I (TnIc) concentrations were measured by immunoassay on a multiparametric analyzer Dimension R×L®; the range for normal values was 0-0.15 μg/L. In plasma and serum, epinephrine (EPI), norepinephrine (NE), cortisol (C), dehydroepiandrosterone sulfate (DH-
EAs), and prolactin (PRL) were assayed in duplicate by radioimmunoassay using commercial kits (LDN, USA; DiaSorin and DSLabs, France). The normal fasting ranges were: < 100 pg/mL for EPI, < 600 pg/mL for NE, 193-690 nmol/L for morning C, 1.33-4.41 were: < 100 pg/mL for EPI, < 600 pg/mL for NE, 193-690 nmol/L for cortisol, 0.06 μg/mL for DHEAs, 0.5 ng/mL for PRL.

Statistical analysis

Statistica Version 6.1 (Statsoft, Tulsa, OK, USA) was used for all analyses. All data were expressed as mean ± SEM. Characteristics of the subjects and their race performances were compared between the long-distance triathlon and 100-km endurance run groups using Student’s t-test. To analyze changes over time and between groups, a two-way, repeated-measures ANOVA was used. If this analysis revealed significant differences, a Newman-Keuls post hoc test was used to identify the specific differences. Pearson product-moment correlations were used to test the relationship between changes in cytokines and concentrations of other blood parameters. P < 0.05 was accepted as significant.

RESULTS

Mean race time

This did not differ significantly between the LD triathlon and the 100-km run groups (504 ± 13 min and 539 ± 10 min respectively).

Effects of races on body weight, physical performance, clinical and biochemical parameters

Compared to T1, mean body weights for either group were not significantly altered. Compared to T1, performances in the vertical jump test were significantly reduced only for runners immediately after and 24 h post-race (R1, R2), and then returned to pre-race values seven days after the race (R3). For the two groups, self-reported muscle and joint soreness were higher immediately after and 24 h post-race (R1, R2), while muscle flexibility was reduced at R1 but only in the runners. Significant, race-related differences were noted for muscle flexibility and performance in the vertical jump at R1 and R2 (table 1).

Inter-relationship between levels of cytokine and biochemical and physical parameters

Table 3 summarizes the correlations between levels of cytokine and those of biochemical parameters (pre-race and race-induced changes). The most significant positive correlations were noted in the triathletes between IL-6, IL-1ra, and IL-10 were significantly increased in both groups, while those of IL-8 only increased in the runners. IL-6, IL-1ra and IL-10 were respectively 43%, 52%, and 78% higher post-race in triathletes as compared to the runners, while IL-8 was 116% lower. These cytokine levels returned to T1 values 24 h and 7 days post-race (figure 1).

Table 1

| Table 1 |
|-------------------|-------------------|-------------------|-------------------|-------------------|
|                  | T1                | R1                | R2                | R3                |
|                  | LD Triathlon      | 100-km Run        | LD Triathlon      | 100-km Run        | LD Triathlon      | 100-km Run        | LD Triathlon      | 100-km Run        |
| Weight (kg)      | 71.8 ± 1.4        | 68.8 ± 1.8        | 69.2 ± 1.3        | 66.8 ± 1.7        | 70.3 ± 1.3        | 67.4 ± 1.9        | 71.9 ± 1.4        | 67.9 ± 1.8        |
| Muscle soreness from Likert scale | 0.8 ± 0.3 | 0.6 ± 0.3 | 3.9 ± 0.3*** | 4.7 ± 0.4*** | 3.3 ± 0.3*** | 3.4 ± 0.2*** | 0.5 ± 0.2 | 0.7 ± 0.3 |
| Joint soreness from Likert scale | 0.1 ± 0.1 | 1.0 ± 0.4 | 2.3 ± 0.5* | 3.1 ± 0.7** | 2.4 ± 0.4* | 2.2 ± 0.6 | 0.5 ± 0.2 | 0.3 ± 0.2 |
| Muscle flexibility | 0.8 ± 0.3 | 1.7 ± 0.7 | 1.0 ± 0.5 | 5.1 ± 1.2*** | 1.0 ± 0.3 | 3.2 ± 0.9 | 1.5 ± 0.5 | 0.1 ± 0.1 |
| Vertical jump (cm) | 50 ± 1 | 44 ± 2 | 48 ± 2 | 29 ± 2*** | 47 ± 2 | 32 ± 3*** | 49 ± 1 | 40 ± 3 |

Values are means ± SE.
LD Triathlon: Long-Distance triathlon; 100-km Run: 100-km endurance Run.
T1: the day before races; R1 after races; R2 and R3: 24 h and 7-day post races.
*p < 0.05, **p < 0.01, ***p < 0.001; versus T1 values.
*p < 0.05, **p < 0.01, ***p < 0.001; between LD Triathlon and 100-km Run.
In addition, HSP72 levels were positively correlated with those of CK and TnIc in the runners (0.47 and 0.49, P < 0.05, respectively), but not in the triathletes (0.08 and 0.26 respectively).

**DISCUSSION**

In agreement with recent reports, we found that while serum levels of three cytokines, IL-6, IL-1ra, and IL-10
rose in response to two race competitions of similar duration, a long-distance triathlon and a 100-km endurance run. TNF-α levels did not change significantly [7, 10, 11]. In contrast, there was an increase in levels of the chemokine IL-8 only after the 100-km run, in addition to increases in levels of creatine kinase (CK) a muscle-specific enzyme considered to be a marker of muscle damage or membrane leakage [23]. In addition, we showed that cytokine levels recovered to pre-race values 24 h and 7 days post-race and that the magnitude of the change in cytokine levels differed significantly between the two races. The highest increases in IL-6, IL-1ra, and IL-10 levels were noted for the triathletes, as were the increases in fatty acids and hormones regulating both the mobilization of substrates for energy and immune responses (cortisol, catecholamines, prolactin and DHEAs). The increase in IL-8, a marker of oxidative stress that we observed for runners, paralleled that in HSP72.

Endurance exercise-induced muscle cell metabolic activity and damage appear to be important triggers of cytokine release, and IL-6 is described as the first cytokine to appear in the circulation [13, 24]. Plasma IL-6 levels have been shown to increase, in an exponential fashion, with exercise as a function of both intensity and duration, the mass of muscle recruited, and the individual’s endurance capacity [13]. It has been demonstrated that IL-6 is released essentially by muscle independently of damage, but in relation to the depletion in glycogen stores and the stimulus for Ca^{2+} ions-liberating muscle contraction [13]. Exercise has also been shown to induce IL-6 production and gene expression in human adipose tissues [14, 15]. Keller et al. [15] have suggested that IL-6 may be released from active tissue beds (adipose tissue and/or skeletal muscle) to increase lipolysis when exercise is prolonged, in order to supply the energy needs. Increased lipolysis by high levels of IL-6 has been demonstrated in both human adipose tissue [25] and rat muscle [16], and the authors suggested that this occurred either directly or indirectly via other hormones such as epinephrine and cortisol.

In the present study, the long distance triathlon led to more lipolysis (as shown by increases in fatty acid levels) and IL-6 release than did the 100-km endurance run, and elicited only slight or no significant elevations in plasma creatine kinase, suggesting that muscle damage was minor. Although our results did not identify the origin of the IL-6 (muscle or adipose tissue), we showed that it is the requirement for energy substrates rather than muscle damage that underlies the release of IL-6 into the circulation [13]. We also showed that the higher degree of lipolysis in the triathletes paralleled increases in IL-6, cortisol, and catecholamines, tending to support a lipolytic action of IL-6 either directly or indirectly [16, 25].

In the runners, the unchanged levels of catecholamines just after the race, two major hormonal lipolytic factors during exercise [26], despite an elevated IL-6 (60-fold above baseline), was consistent with a role for IL-6 in lipolysis in order to meet energy demand during this extreme endurance exercise. In agreement with other studies, plasma glucose concentrations remained unchanged after the two races [11], indicating a sufficient supply (endogenous and exogenous) of carbohydrates during the races. Based on earlier studies showing depleted glycogen stores in muscle during prolonged strenuous exercise despite ingestion of carbohydrates [27], we surmised that the increases in IL-6 observed after the two races were influenced by depletion

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**Table 3**

Correlations between circulating levels of cytokines and biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Runners (n = 11)</th>
<th>Triathletes (n = 12)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0.76*</td>
<td>0.83*</td>
<td>FFA</td>
</tr>
<tr>
<td></td>
<td>0.61*</td>
<td>0.02</td>
<td>CK</td>
</tr>
<tr>
<td></td>
<td>0.47*</td>
<td>0.14</td>
<td>TnIc</td>
</tr>
<tr>
<td></td>
<td>0.86*</td>
<td>0.82*</td>
<td>HSP72</td>
</tr>
<tr>
<td></td>
<td>0.45*</td>
<td>0.74*</td>
<td>PRL</td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td>0.78*</td>
<td>DHEAs</td>
</tr>
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<td>IL-1ra</td>
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<td>0.87*</td>
<td>FFA</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.10</td>
<td>CK</td>
</tr>
<tr>
<td></td>
<td>0.72*</td>
<td>0.13</td>
<td>TnIc</td>
</tr>
<tr>
<td></td>
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<td>0.73*</td>
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<td></td>
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<td>0.74*</td>
<td>PRL</td>
</tr>
<tr>
<td></td>
<td>-0.06</td>
<td>0.70*</td>
<td>DHEAs</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.53*</td>
<td>0.83*</td>
<td>FFA</td>
</tr>
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<td>0.06</td>
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<td>0.28</td>
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<td>0.82*</td>
<td>HSP72</td>
</tr>
<tr>
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<td>PRL</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.66*</td>
<td>DHEAs</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.46*</td>
<td>0.61*</td>
<td>FFA</td>
</tr>
<tr>
<td></td>
<td>0.36*</td>
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<td>CK</td>
</tr>
<tr>
<td></td>
<td>0.40*</td>
<td>0.12</td>
<td>TnIc</td>
</tr>
<tr>
<td></td>
<td>0.92*</td>
<td>0.49*</td>
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<td></td>
<td>0.42*</td>
<td>0.60*</td>
<td>PRL</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.57*</td>
<td>DHEAs</td>
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</table>

* Significant correlations (p < 0.05) obtained using Pearson’s product moment coefficient.
of muscle glycogen rather than by blood glucose concentration. However, we could not rule out an influence of an increase in muscular Ca\textsuperscript{2+} content [2], which is a potent signalling factor for IL-6 transcription in muscle.

We have shown here that the increases in the main anti-inflammatory cytokine IL-10, and the IL-1 cytokine inhibitor (IL-1ra) were higher for the triathletes than for the runners, and paralleled the changes observed for IL-6. As IL-6 has been shown to induce these two anti-inflammatory cytokines in humans, as well as the anti-inflammatory hormone cortisol [28], the larger increase in IL-6 in our triathletes would support an inhibitory role for this cytokine in inflammation.

However, we noted significant anti-inflammatory responses during the long distance triathlon, which could be triggered independently of IL-6. Indeed, levels of prolactin and DHEAs, two immune-enhancing hormones [29, 30], were only increased in the triathletes and were correlated with levels of the two main anti-inflammatory cytokines, IL-10 and IL-1ra. After acute, maximal, aerobic exercise, an immunostimulatory action of prolactin has been proposed, as its plasma concentration and its receptor expression on human B lymphocytes increase in line with circulating B lymphocytes [31]. In this respect, we have observed depressed cellular immunity after intense training concomitant with reduced levels of prolactin and DHEAs [32, 33].

In comparison to the changes we observed for IL-6 and its related cytokines IL-1ra and IL-10, levels of the chemokine IL-8 were increased, but only in the runners who also exhibited increased CK levels and reductions in both muscle flexibility and physical performance. In addition, in the runners there were positive correlations between IL-8 and markers of muscular damage, CK and cardiac troponin I (TnIc, the specific cardiac marker). IL-8, a chemoattractant protein, is produced by monocytes and macrophages and many other tissues [34]. IL-8 has been reported to trigger the adhesion of monocytes to vascular endothelium under flow conditions and stimulation by mechanical stretch, and to play a role in angiogenesis [34, 35].

A marked increase in circulating IL-8 has been reported after prolonged endurance exercise (marathon, half-marathon, and 160-km race) involving a substantial eccentric component and muscle damage [10, 12, 18, 36]. Two studies in humans have shown increased muscle IL-8 expression, but no change in plasma levels during a prolonged, concentric exercise which was not associated with an inflammatory response [37, 38]. Akerstrom et al. [37] related the systemic increase in IL-8 observed during exercise with an eccentric component, to an inflammatory response, while they suggested that muscle-derived IL-8 may play a local role in angiogenesis. Although, we could not determine the origin of IL-8 release, our results supported a link with inflammation related to the high degree of mechanical restraint during the 100-km run, particularly on muscle and vascular endothelium. In addition, we suggested that the higher cortisol levels triggered by the long distance triathlon inhibited release of IL-8 [39].

The pronounced rise, especially of IL-8, after a half-marathon is indicative of oxidative stress, which may be responsible for the intracellular HSP70 expression in immune cells [36]. The ubiquitous HSP70 family of proteins, which possess a high degree of conservation, plays a role in cellular repair processes in response to various stressors (temperature, ischemia, protein degradation, hypoxia, acidosis, reduced glucose availability, oxyradical formation, and increased intracellular Ca\textsuperscript{2+}) [40-42]. Long-lasting, competitive endurance exercise has been found to result in a pronounced release of HSP72 in the blood circulation, although it is not completely clear from which tissue it originates [43]. Indeed, several sources for circulating HSP72 during exercise have been described, such as damaged muscle cells, leukocytes, liver, heart, and brain. Several exercise conditions may influence its release such as ambient temperature, training status and age of subjects [43]. We observed increased HSP72 levels at the end of both races, but also a strong, race-related difference with levels being 173% higher in the runners than in the triathletes. At the end of the 100-km run, HSP72 levels in plasma were similar to those measured soon after a marathon [43]. In addition, HSP72 levels in runners correlated highly with those of IL-8 (0.92), and also with markers of muscular damage CK and TnIc (0.47 and 0.49 respectively) suggesting that muscle damage and oxidative stress are capable of initiating a heat shock response.

The difference in the HSP response between the two races could be related to: (i) their respective characteristics such as part of eccentric/concentric muscle contractions, exercise intensity, age and training status of subjects, (ii) race conditions such as ambient temperature and weather, (iii) influence of the water immersion during swimming in the triathlon. In conclusion, comparative results from two different endurance events (a long distance triathlon and a 100-km run) are consistent with the notion of IL-6 acting in a hormone-like manner in response to energy demand during protracted exercise. In addition, we showed that a high degree of mechanical restraint on the lower limbs triggered a systemic and transient release of IL-8, a chemoattractant cytokine playing a role in vascular inflammation. Extreme exercise initiated a heat shock response dependent upon the magnitude of the disruption to cellular homeostasis. Taken together, the changes we observed after extreme exercise support a relationship between systemic cytokine responses and metabolic and inflammatory processes.

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