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Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery

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We investigated changes in a large battery of pro- and anti-inflammatory cytokines in elite female soccer players following two 90-min games separated by a 72-h active or passive recovery. Blood samples were taken from 10 players before, within 15–20 min, 21, 45 and 69 h after the first game and within 15–20 min after the second game. The leukocyte count was analyzed, together with several plasma pro- and anti-inflammatory cytokines, using a multiplex bead array system. After the first and second game, the total leukocytes and neutrophils increased significantly. Likewise, increases ($P < 0.05$) in pro-inflammatory cytokines [interleukin (IL)-12, tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), IL-17], chemokines [monocyte chemotactic protein-1 (MCP-1), IL-8 and monokine induced by gamma interferon (MIG)], anti-inflammatory cytokines (IL-2R, IL-4, IL-5, IL-7, IL-10, IL-13, INF-α) and the mixed cytokine IL-6 were observed. Leukocyte and cytokine levels were normalized within 21 h. Active recovery (low-intensity exercises) did not affect the cytokine responses. A dampened cytokine response was observed after the second game as only IL-12, IL-6, MCP-1, IL-8 and MIG increased ($P < 0.05$). In conclusion, a robust pro- and anti-inflammatory cytokine response occurs after the first but not the second soccer game. The implications of the dampened cytokine response in female players after the second game are unknown.

In soccer, players have to perform weekly in comparison with some individual sports comprising only a few large competitions per year. A competitive soccer season includes one to two games per week in addition to several training sessions. In women competitive soccer tournaments, games are played even more frequently, allowing for only 2 days of recovery between games. Competitive soccer affects the musculoskeletal, nervous, immune and metabolic systems (Reilly & Ekblom, 2005). We have previously reported that several neuromuscular parameters are affected for up to 69 h after an elite female soccer game (Andersson et al., 2008). For instance, the isokinetic knee strength was reduced up to 45 h after a first game and jump performance did not recover before the start of a second game played 72 h after the first one. Furthermore, changes in CK levels and perceived muscle soreness lasted up to 69 h after the first soccer game (Andersson et al., 2008). These findings show that several physiological parameters are disturbed after a soccer game. This, in turn, may trigger a systemic immune response that leads to the secretion of cytokines by inflammatory cells (Ostrowski et al., 1999).

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Few studies are available on the cytokine response following soccer games (Bishop et al., 2002; Ascensão et al., 2008; Ispirlidis et al., 2008). An increased leukocyte count and increased levels of interleukin (IL)-6 and tumor necrosis factor (TNF-α) have been shown in male soccer players (Bishop et al., 2002; Ascensão et al., 2008; Ispirlidis et al., 2008). It has also been shown that 5 days of consecutive soccer training in youth male players decreased T and B cell numbers, possibly affecting their capability to activate the immune system and resist infections (Malm et al., 2004a).

Cytokines are potent intercellular signaling molecules that regulate the inflammation response (Suzuki et al., 2003). It has been shown that the plasma pro-inflammatory cytokine response after endurance exercise can be balanced by the production of anti-inflammatory cytokines (Ostrowski et al., 1999). To our knowledge, a comprehensive evaluation of the cytokine response following a soccer game is lacking and the time course of the pro- and anti-inflammatory cytokine responses between two games has never been addressed. Furthermore, the cytokine response has never been documented in female soccer players. Consequently, a thorough evaluation of the inflammatory response in female soccer players would provide new insights into the physiological alterations occurring during soccer games and the subsequent recovery periods.

Given the above rational, the aims of this study were to determine (a) changes in the plasma levels of a large battery of pro- and anti-inflammatory cytokines within 15–20 min after a first and a second elite female soccer game and (b) the time course of the pro- and anti-inflammatory cytokine recovery during the 72-h period separating the two games. Furthermore, as this study is part of a project aiming to assess the effects of active recovery training during the period between the two games, we have also evaluated whether the cytokine response is affected by active recovery training.

Materials and methods

Subjects

Twenty-two elite female soccer players played two 90-min international friendly games separated by 72 h. The subjects played in the highest division in Sweden and Norway and were experienced soccer players. The games were conducted in the middle of the soccer season when the players are accustomed to playing soccer games. The games were friendly and organized for the needs of this study. Before the commencement of the study, the players had rested at least 3 days from games and 2 days from soccer training. All players played the whole games (90 min) in both matches. This study is part of a larger research project aiming to investigate the effects of an active recovery training program on the recovery of neuromuscular and biochemical parameters (Andersson et al., 2008). The participants were randomly assigned to an active recovery training group or a passive control group during the recovery period separating the two soccer games. For the purpose of this study, two forwards, three midfielders and five defenders were randomly chosen to be included in the cytokine analysis (height 167 ± 6 cm, weight 64 ± 6 kg, age 23 ± 4 years, heart rate $(HR)_{peak}$ 198 ± 2 bpm and VO_{2max} 54 ± 3 mL/kg/min). In total, 10 subjects (active recovery group $n = 5$, passive group $n = 5$) were included in the cytokine analysis. Two days before the commencement of the study, all subjects performed a maximal oxygen consumption test running on a treadmill using a protocol described elsewhere (Raastad et al., 1997). Briefly, after a warm-up period the subjects ran with a 3° inclination starting at 9 km/h. The speed was increased every minute by 1 km/h until the subjects reached exhaustion. The oxygen uptake was measured using an online system and HR was recorded at 5-s intervals during the entire protocol by a Polar 610i monitor (Polar Electro OY, Kempele, Finland). Peak HR was recorded. The players were informed of the experimental procedures and possible discomforts associated with the study before they gave their written informed consent to participate. The study was conducted according to the Declaration of Helsinki and approved by the Regional Ethics Committee of Uppsala, Sweden (Dnr 2004: M-364).

Experimental design

The study was carried out during a time course of 5 days where two 90 min games were separated by a 72-h recovery. Heart rate was recorded to evaluate the aerobic work load during the games. The players wore a HR monitor around their chest and data were continuously collected every 5 s during the game (Polar Team System, Polar Electro OY). During the recovery period between the games, one group (active recovery group) performed low intensive training for 1 h consisting of submaximal cycling at 60% HR_{peak} and low-intensity resistance training at < 50% 1 RM, while the other group (passive recovery group) rested (for a detailed description, see Andersson et al., 2008). During the whole experiment, blood was sampled at 10:00 hours every day and within 15–20 min after the games. Blood samples were obtained 1 h before (baseline) and within 15–20 min, 21, 45 and 69 h after the first game and within 15–20 min after the second game. A total of 25 cytokines were analyzed in the plasma.

Diet

Food intake was standardized during the whole study period. All players ate together and the meal plan was formulated by a nutritionist. The composition of the meals was developed using a national food database ("Food on data" 4.3 LKH, Norway), and carbohydrate (CHO) and protein intake were adjusted to the players' body weight to meet the recommendations for daily recovery in players participating in moderate training (intake of ≥ 6 g/body weight CHO and ≥ 1.2 g/kg body weight protein) (Maughan et al., 2004). In addition, the players were instructed to drink a sports drink during the games providing approximately 30–60 g CHO/h (Maxim Energy™, Maxim International A/S Ishøj, Denmark). The sports drink did not contain vitamin C. After the blood sample was taken at the end of the game each player had a CHO intake of 1 g/kg body weight within 30 min to ensure optimal recovery (banana, yoghurt and sports drink) (Maughan et al., 2004).

Blood sampling

Blood was drawn into 4 mL EDTA vacutainers and a 9 mL vacutainer tube. The blood in the 9 mL vacutainer coagulated at room temperature for 30–45 min. Serum was pipetted off and stored in Eppendorf tubes at −80 °C until analysis. One of the EDTA tubes was centrifuged at 1500 *g* at 4 °C for 15 min to obtain plasma. Plasma was further centrifuged for 5 min at 11 000 *g* at 4 °C: the supernatant was stored at −80 °C until analysis. Hemoglobin and hematocrit were analyzed with a Sysmex K-1000 (TOA Medical Electronics Co. Ltd., Kobe, Japan). Glucose was analyzed in serum with standard routine measurements in a Modular P® Analyzer (Hitachi, Tokyo, Japan).

Leukocyte differential counts

Leukocytes numbers were analyzed with a Sysmex K-1000 (TOA Medical Electronics Co. Ltd.). Coefficients of variation (CV) for neutrophils and lymphocytes counts are <4%. Leukocytes in EDTA whole blood were counted as the total number of leukocytes [white blood cells (WBC)], neutrophils, lymphocytes and mixed cells. Mixed cells are monocytes, basophil and eosinophil granolucytes.

Cytokine analyses

IL-1β, IL-1Ra, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, INF-γ, INF-α, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, TNF-α, granulocyte monocyte colony-stimulating factor (GM-CSF), inducible protein 10 (IP-10), MIP-1α,β, monocyte chemotactic protein-1 (MCP-1), monokine induced by gamma interferon (MIG), Eotaxin and regulated upon activation, normal T cell expressed and secreted (RANTES) were measured in the plasma using a validated sandwich immunoassay-based protein array system commercial kit (Biosource International, kit no. LHC009, Camarillo, California, USA). Cytokine detection was

performed according to the manufacturer's instruction, with assay diluents as a blank. Calibration standards were prepared in the assay buffer. In brief, antibody-coupled microspheres specific for the different cytokines were incubated with plasma. Antigen binding was then detected after incubation with biotinylated detector antibodies. Detection was performed using a flow-based dual laser detector with a real-time digital signal. The Luminex 100 IS instrument (Biosource, Nivelles, Belgium) with the Star Station acquisition program (v2 Applied Cytometry Systems, Sheffield, UK) was used to process the data. According to the manufacturer, both the intra-assay variation and the inter-assay variation for all antigens measured by the multiplex system are below 10%. Furthermore, the sensitivity of multiplex bead array assays for the detection of soluble cytokines from various manufacturers has been compared (Khan et al., 2004). First, Bead array and enzyme-linked immunosorbent assay (ELISA) values appeared to be comparable between the manufacturers (Khan et al., 2004). Second, the minimal detection range for the kit used in our study (Biosource kit, Biosource international) was comparable and even several fold higher than the other kits (Khan et al., 2004). Third, the sensitivity limits are equivalent to those of most ELISAs (Martins et al., 2002). All samples were run in duplicate samples, according to the manufacturer's recommendations.

Statistical analyses

For normally distributed data, a two-way (group × time) repeated measure ANOVA was performed, followed by Tukey's *post hoc* test. Additionally, for data showing only time effects, Dunnett's *post hoc* test was used to detect significant differences from baseline values. For data that did not meet the assumption of normality, Friedman's non-parametric test was used, followed by a Dunn *post hoc* test. A Student's paired *t*-test was used to test single comparisons, such as the difference between the samples obtained within 15–20 min pre- to post-game data. *P*-values below 0.05 were considered statistically significant. Data are presented as means and standard errors of the mean (SEM). The Statistical Package for the Social Sciences (SPSS Inc., version 12.0) and Statistica (StatSoft Inc., version 7.0) were used for the analyses.

Results

Effects of active recovery training on the cytokine response

In the present study, we found no differences in the cytokine response between the active recovery group and the passive group at any time point. Importantly, there were significant time effects on the inflammatory cytokine response. For this reason, changes in plasma cytokines at different time points of the experiments are presented as a mean for both groups (10 players).

Physiological responses during the games

We have previously shown that the amount of high-intensity running (1.09 ± 0.2 vs 1.11 ± 0.1 km), plasma CK levels (323 ± 36 vs 376 ± 53 U/L), neuromuscular changes and fluid intake were similar after both games (Andersson et al., 2008). Here, we report that the mean HR was higher ($P < 0.05$) in the second game (168 ± 2 bpm) compared with the first game (162 ± 2 bpm) but the peak HR during both games was similar (190 ± 2 bpm in the second game vs 187 ± 2 bpm in the first game). The hemoglobin levels ($\sim 13.3 \pm 0.2$ g/dL) remained unchanged after both games. Glucose levels before the first and the second game were similar ($\sim 4.0 \pm 0.2$ mmol/L) and increased similarly (to $\sim 5.3 \pm 0.2$ mmol/L) after the first and second game (Fig. 1). Furthermore, we noted similar weather conditions when the two matches were played (light rain and ~ 12 °C).

Leukocyte count response

Baseline values for total leukocyte ($5.3 \pm 0.4 \times 10^3$ cells/mL) and neutrophil ($3.0 \pm 0.3 \times 10^3$ cells/mL) counts were within normal reference intervals. Total leukocytes and especially neutrophil counts significantly increased following the first game and were normalized within 21 h after the first game (Fig. 2). Immediately after the second game, the total leukocyte and neutrophil counts increased with the same magnitude as after the first game (Fig. 2). Lymphocyte ($1.8 \pm 0.1 \times 10^3$ cells/mL) and mixed leukocyte ($0.5 \pm 0.0 \times 10^3$ cells/mL) counts remained unchanged after both the first and the second game (Fig. 2).

Cytokine response

Immediately after the first game, a significant elevation occurred in the plasma concentration of twelve cytokines: IL-12, TNF-α, INF-γ, IL-6, IL-2R, IL-4, IL-5, IL-7, IL-10, IL-13, IL-17 and INF-α [Fig. 3(a)–(c)]. A significant increase in the chemokines IL-8, MIG and MCP-1 also occurred after the first game [Fig. 3(d)]. The levels of IL-1β, IL-1Ra, IL-2, IL-15, GM–CSF, IP-10, MIP-1-α and -β, Eotaxin and RANTES were not significantly affected throughout the study period (Tables 1–3). For the analysis of IL-15 and IL-17, the levels were low or undetectable in three (IL-15)

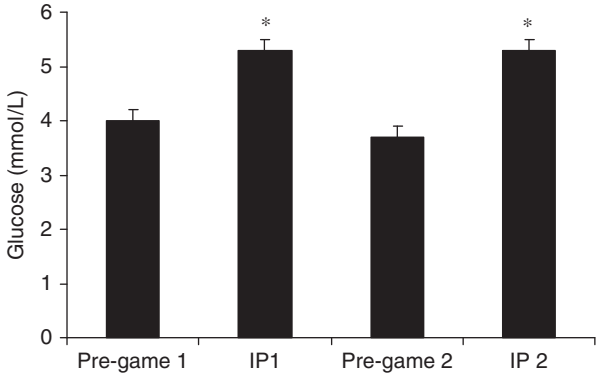

Fig. 1. Plasma glucose in elite female players before and after two 90-min soccer games. Values are mean ± SE. IP, immediately post-game; *significantly higher than pre-game values ($P < 0.05$). [Correction have been made to figure after initial online publication.]

respective four (IL-17) subjects at all time points. All cytokines and chemokines were normalized within 21 h after the first game and remained at baseline levels until the beginning of the second game (Tables 1–3). The cytokine response following the second game differed from that of the first game. Only two cytokines, IL-12 and IL-6, significantly increased after the second game whereas the same chemokines, IL-8, MIG and MCP-1, increased after the second game. The magnitude of changes in the two cytokines (IL-6 and IL-12) and chemokines (IL-8, MIG and MCP-1) was similar after the first and second games [Fig. 3(b), (c) and (d)].

Discussion

The present study provides a thorough evaluation of the plasma inflammatory cytokine response in elite female soccer players and establishes a time course of cytokine changes during a period consisting of two games separated by a 72-h recovery. We report for the first time that a female soccer game induces a robust, but transient, increase in a large number of both pro- and anti-inflammatory cytokines and inflammatory cells, and that a dampened cytokine response is observed when a second game is played 72 h after the first game. Moreover, IL-6, IL-12, IL-8, MCP-1 and MIG increased following both games, indicating a more pronounced pro-inflammatory response by the end of the second game. Furthermore, the cytokine response patterns did not differ between active and passive recovery. Although the number of subjects in the active and passive recovery groups was low, the lack of effect of the recovery regime on the cytokine response is in agreement with our previous findings showing that active recovery failed to influence neuromuscular parameters, CK and urea levels and perceived muscle soreness (Andersson et al., 2008).

High-intensity exercise induces a cardiovascular stress that increases the levels of stress hormones. The increase in catecholamines and cortisol, together with changes in metabolic activities, the occurrence of membrane disruptions in muscle cells and increased free radical production (Suzuki et al., 2000; Steensberg et al., 2003; Nieman et al., 2005), can lead to the activation of the immune system, such as the release of several pro-inflammatory cytokines and chemokines. The pro-inflammatory cytokines and chemokines are believed to activate neutrophils and anti-inflammatory cytokines. The anti-inflammatory cytokines would attenuate inflammation by restricting inflammatory cytokine production, up-regulating their soluble antagonist-binding proteins and suppressing inflammatory cell activity (Kluth & Rees, 1996).

The increase in the total number of circulating leukocytes occurring by the end of both games was expected, and similar to what has been reported in male players (Malm et al., 2004b; Ascensão et al., 2008; Ispirlidis et al., 2008). Consequently, there is no apparent sex difference in the leukocyte response after soccer games. The increase in the leukocyte count was primarily explained by the elevation of

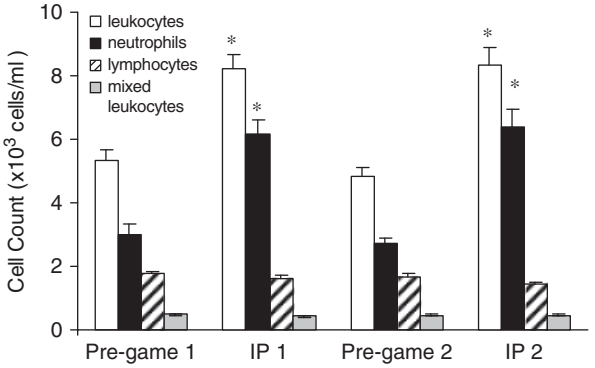

Fig. 2. Plasma leukocyte, neutrophil, lymphocyte and mixed cell counts in elite female players before and after two 90-min soccer games. Values are mean ± SE: IP, immediately post-game; *significantly higher than pre-game values ($P<0.05$). [Correction have been made to figure after initial online publication.]

Table 1. Pro-inflammatory cytokine response in elite female players before and after two soccer games separated by 72 h recovery

Pro-inflammatory cytokines (pg/mL)	Group	*n*	Baseline	IP game 1	Day 2 (21 h)	Day 3 (45 h)	Pre-game 2 (69 h)	IP game 2
IL-2	Active	5	10 ± 5	12 ± 5	10 ± 5	8 ± 4	10 ± 5	9 ± 4
	Passive	5	7 ± 3	7 ± 3	6 ± 3	6 ± 3	4 ± 2	5 ± 2
IL-12	Active	5	90 ± 14	124 ± 25*	116 ± 22	109 ± 20	113 ± 24	130 ± 23*
	Passive	5	109 ± 20	153 ± 28*	126 ± 33	126 ± 29	91 ± 11	135 ± 15*
TNF-α	Active	5	7 ± 1	24 ± 10*	17 ± 4	8 ± 1	10 ± 2	9 ± 1
	Passive	5	9 ± 1	25 ± 3*	14 ± 3	8 ± 1	15 ± 5	17 ± 2
IL-17	Active	5	0.2 ± 0.2	3.3 ± 1.3*	3.5 ± 2.1	1.0 ± 0.5	0.8 ± 0.4	0.3 ± 0.2
	Passive	5	0.2 ± 0.1	4.8 ± 1.8*	1.5 ± 1.0	1.4 ± 0.8	0.4 ± 0.2	1.8 ± 1.2
INF-γ	Active	5	2.9 ± 1.4	13.3 ± 2.9*	7.8 ± 2.6	1.4 ± 0.8	3.0 ± 1.4	8.6 ± 3.1
	Passive	5	2.1 ± 1.2	12.3 ± 4.8*	5.9 ± 3.6	2.1 ± 1.5	8.4 ± 3.5	5.9 ± 2.6

Values are mean ± SE.

*Significantly higher than baseline values $P<0.05$.

IP, immediately post games; TNF-α, tumor necrosis factor-α; INF-γ, Interferon-γ.

Table 2. Chemokine response in elite female players before and after two soccer games separated by a 72 h recovery

Chemokines (pg/mL)	Group	*n*	Baseline	IP game 1	Day 2 (21 h)	Day 3 (45 h)	Pre-game 2 (69 h)	IP game 2
IL-8	Active	5	0.5 ± 0.1	1.4 ± 0.5*	0.8 ± 0.4	0.5 ± 0.3	0.6 ± 0.2	1.4 ± 0.4*
	Passive	5	0.4 ± 0.1	2.4 ± 0.3*	0.4 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	1.6 ± 0.5*
MIG	Active	5	4 ± 1	12 ± 3*	9 ± 3	9 ± 4	16 ± 6	22 ± 7*
	Passive	5	11 ± 4	19 ± 3*	11 ± 2	10 ± 3	11 ± 2	15 ± 2*
MCP-1	Active	5	64 ± 4	106 ± 12*	81 ± 11	74 ± 9	76 ± 10	117 ± 13*
	Passive	5	83 ± 4	143 ± 9*	86 ± 7	84 ± 7	75 ± 7	148 ± 11*
MIP-α	Active	5	18 ± 5	27 ± 7	21 ± 6	20 ± 3	20 ± 5	17 ± 3
	Passive	5	15 ± 5	24 ± 4	19 ± 6	13 ± 5	8 ± 3	17 ± 2
MIP-β	Active	5	27 ± 7	37 ± 9	36 ± 9	25 ± 9	36 ± 11	29 ± 6
	Passive	5	31 ± 5	49 ± 3	23 ± 5	25 ± 5	21 ± 8	36 ± 8
IP-10	Active	5	21 ± 1	17 ± 1	24 ± 2	28 ± 3	41 ± 11	35 ± 11
	Passive	5	20 ± 3	17 ± 2	23 ± 2	22 ± 2	20 ± 3	19 ± 2
Eotaxin	Active	5	28 ± 2	31 ± 4	32 ± 5	28 ± 3	30 ± 3	30 ± 2
	Passive	5	35 ± 2	37 ± 1	34 ± 4	35 ± 4	30 ± 2	39 ± 3
RANTES	Active	5	8344 ± 2324	6164 ± 1394	5250 ± 1539	5049 ± 1101	6692 ± 2864	4087 ± 1236
	Passive	5	12 085 ± 2154	5150 ± 1548	7249 ± 2070	11 888 ± 1848	7429 ± 1891	7626 ± 1394
GM-CSF	Active	5	8 ± 6	21 ± 9	11 ± 4	8 ± 2	9 ± 3	9 ± 6
	Passive	5	7 ± 3	17 ± 4	6 ± 1	4 ± 1	5 ± 4	11 ± 4

Values are mean ± SE.

*Significantly higher than baseline values $P<0.05$.

IP, immediately post games; IL, interleukin; MCP-1; monocyte chemotactic protein-1; RANTES, regulated upon activation, normal T cell expressed and secreted; GM-CSF, granulocyte monocyte colony-stimulating factor; IP-10, inducible protein 10; MIG, monokine induced by gamma interferon.

Table 3. Anti-inflammatory cytokine response in elite female players before and after two soccer games separated by 72 h recovery

Anti-inflammatory cytokines (pg/ml)	Group	*n*	Baseline	IP game 1	Day 2 (21 h)	Day 3 (45 h)	Pre-game 2 (69 h)	IP game 2
IL-1β	Active	5	19 ± 6	21 ± 8	24 ± 9	23 ± 7	20 ± 6	18 ± 8
	Passive	5	23 ± 10	22 ± 10	27 ± 8	21 ± 11	17 ± 10	18 ± 7
IL-1ra	Active	5	188 ± 48	236 ± 67	221 ± 60	201 ± 52	176 ± 41	206 ± 47
	Passive	5	221 ± 8	191 ± 58	223 ± 31	224 ± 34	154 ± 27	181 ± 36
IL-2R	Active	5	176 ± 22	278 ± 57*	210 ± 30	190 ± 25	205 ± 27	206 ± 24
	Passive	5	145 ± 19	177 ± 23*	138 ± 15	134 ± 21	133 ± 27	148 ± 22
IL-4	Active	5	3.7 ± 1.1	12.5 ± 3.4*	9.0 ± 2.4	5.8 ± 2.1	7.2 ± 2.3	6.3 ± 1.2
	Passive	5	7.6 ± 1.0	19.8 ± 4.8*	7.4 ± 2.2	3.9 ± 0.6	6.6 ± 2.8	12.0 ± 2.5
IL-5	Active	5	1.2 ± 0.3	2.7 ± 1.0*	2.2 ± 0.7	1.0 ± 0.4	1.1 ± 0.4	1.4 ± 0.3
	Passive	5	1.0 ± 0.3	2.5 ± 0.2*	1.2 ± 0.4	0.7 ± 0.3	0.8 ± 0.4	1.8 ± 0.4
IL-7	Active	5	1.7 ± 0.6	8.6 ± 3.2*	6.0 ± 2.6	3.0 ± 1.1	4.2 ± 1.5	2.1 ± 1.0
	Passive	5	5.0 ± 1.2	10.5 ± 1.6*	5.6 ± 2.3	1.8 ± 0.3	4.7 ± 1.5	7.3 ± 2.1
IL-10	Active	5	25 ± 6.8	41 ± 15*	40 ± 9	26 ± 6	32 ± 8	36 ± 8
	Passive	5	40 ± 14	49 ± 15*	46 ± 17	40 ± 16	32 ± 11	43 ± 13
IL-13	Active	5	14 ± 5	44 ± 13*	33 ± 11	16 ± 5	26 ± 12	28 ± 5
	Passive	5	21 ± 5	48 ± 11*	20 ± 7	16 ± 4	21 ± 10	31 ± 5
INF-α	Active	5	8 ± 5	29 ± 10*	23 ± 15	13 ± 8	22 ± 10	14 ± 6
	Passive	5	21 ± 4	55 ± 18*	28 ± 6	7 ± 3	21 ± 7	28 ± 8
IL-15	Active	5	5.4 ± 1.9	8.1 ± 3.1	6.8 ± 1.8	5.9 ± 1.5	4.4 ± 1.3	3.7 ± 0.9
	Passive	5	3.6 ± 1.2	5.2 ± 2.0	4.4 ± 2.1	3.9 ± 1.7	2.5 ± 1.5	5.3 ± 2.9
IL-6 (mixed)	Active	5	2.1 ± 0.8	11.3 ± 3.7*	6.0 ± 3.1	3.5 ± 0.9	3.9 ± 1.9	10.6 ± 2.8*
	Passive	5	5.4 ± 1.5	9.8 ± 1.5*	6.1 ± 2.1	4.2 ± 0.8	4.3 ± 2.5	10.1 ± 3.1*

Values are mean ± SE.

*Significantly higher than baseline values $P<0.05$.

IP, immediately post games; IL, interleukin; INF-α, Interferon-α.

neutrophils, as lymphocytes and mixed cell counts were unchanged after the games (Fig. 2). The increase in leukocytes was brief as it was normalized within 21 h after the game, which is also in agreement with data reported in male players (Ascensão et al., 2008; Ispirlidis et al., 2008).

As consistently reported in response to various exercise protocols (Ostrowski et al., 1998; Suzuki et al., 2000; Bishop et al., 2002; Ispirlidis et al., 2008), we observed an increase in the mixed cytokine IL-6 (2.8-fold) following the soccer game. Our findings also indicate that an elite female soccer game induces a robust inflammatory cytokine response, including an increase in several pro- and anti-inflammatory cytokines. The pro-inflammatory cytokine response included increases in TNF-α, IL-12, INF-γ and IL-17

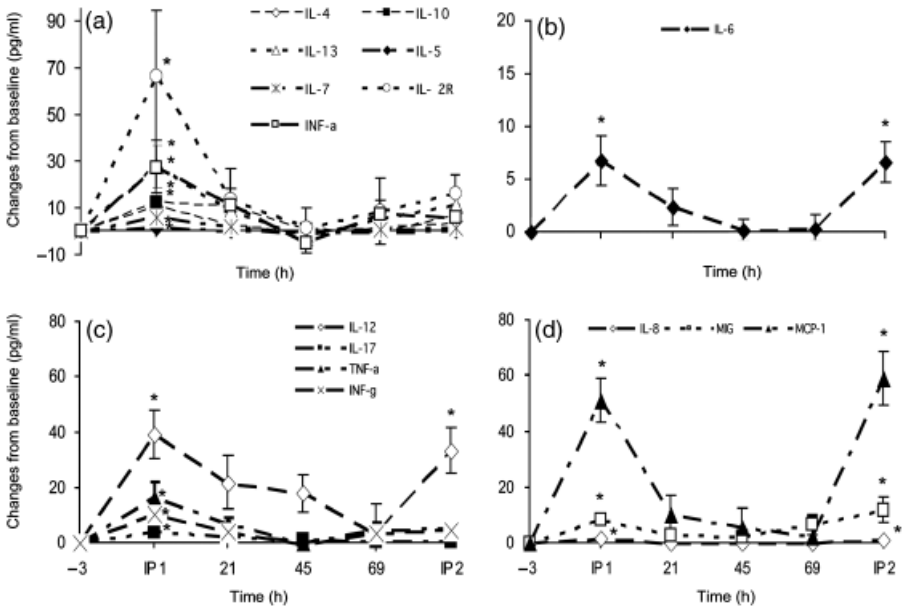

Fig. 3. (a–d) Plasma inflammatory cytokine response in female elite soccer players following two games separated by a 72-h recovery. Values are absolute change (mean ± SE) from baseline: (a) anti-inflammatory cytokines, (b) interleukin (IL)-6, (c) pro-inflammatory cytokines and (d) chemokines. IP, immediately post-games; *significantly higher than baseline ($P < 0.05$).

and the chemokines IL-8, MCP-1 and MIG. In parallel, we observed a robust increase in the number of cytokines with primarily anti-inflammatory functions (IL-10, IL-4, IL-13, INF-α, IL-5, IL-7 and IL-2R) following the first game. Altogether, these results clearly illustrate a response pattern where increases in pro-inflammatory cytokines are accompanied by increases in anti-inflammatory cytokines following the game. This observation is in agreement with the hypothesis suggesting a balance between the pro- and the anti-inflammatory response following exercise (Ostrowski et al., 1999; Zaldivar et al., 2006).

The large cytokine and leukocyte response after the first soccer game may also be explained by interactions between cytokines. For example, it has been suggested that IL-6 plays a central role in the cytokine cascade (Steensberg et al., 2003) as increases in IL-6 have been shown to precede that of other cytokines (Ostrowski et al., 1999). It is also known that IL-6 and IL-8 can attract neutrophils (Schröder, 2000). Therefore, the increase in the neutrophil count immediately after the first and the second game may be linked to the increases in IL-6 and IL-8. Furthermore, IL-6 may also up-regulate anti-inflammatory cytokines IL-10 and IL-ra (Steensberg et al., 2003), which may have contributed to the increase in IL-10 seen immediately after the first soccer game. Moreover, increases in IL-10 may be involved in the up-regulation of the anti-inflammatory cytokines IL-4 and IL-13 (Elenkov & Chrousos, 2002). Likewise, the pro-inflammatory cytokine IL-12 may have stimulated increases in TNF-α and IFN-γ (Elenkov & Chrousos, 2002). It has also been reported that MIG can be regulated by the interferons INF-α, INF-β and INF-γ and other cytokines, such as IL-12 (Strieter et al., 2005).

All pro- and anti-inflammatory cytokines reverted to baseline values within 21 h following the first game. The robust change in the cytokine response within 15–20 min after the game is similar to what has been shown in different exercise protocols. A normalization of the cytokine response 1–2 h after endurance exercise has been reported previously (Zaldivar et al., 2006). This fast counter-regulation might indicate that the normalization of the cytokine levels after exercise occurs within a few hours after the exercise. Likewise, Weinstock et al. (1997) showed a fast counter-regulation of the cytokine response within a few hours after the exercise and a normalization of cytokine levels at 20 h (Weinstock et al., 1997).

Unexpectedly, the cytokine response following the second game clearly indicates a dampened response that markedly differed from that seen in the first game. Only two cytokines (IL-12 and IL-6) and three chemokines (IL-8, MIG and MCP-1) increased after the second game. Interestingly, the amplitude of the pro-inflammatory cytokine response (IL-12, IL-8, MIG and MCP-1) was more pronounced compared with the anti-inflammatory reaction (when taking into account the anti-inflammatory properties of the mixed cytokine IL-6) following the second game.

There are several possible hypotheses behind the dampened cytokine response following the second game. First, it has been shown that the cytokine response during exercise is dependent on the exercise intensity and duration (Pedersen, 2000). However,

our results show that the physical load placed on the players after the first and second games is comparable. We have previously reported that both games induced similar reductions in the performance parameters and elevations in plasma CK levels as seen after the first game (Andersson et al., 2008). Thus, in our study, the dampened cytokine response cannot be explained by a lower match intensity in the second game. In fact, the match intensity in the second game was slightly higher than in the first game. Second, it has been reported that CHO loading attenuates the cytokine response following exercise (Bishop et al., 2002; Chan et al., 2003) and that CHO availability may influence the magnitude of post-exercise disturbance in markers of immune function (Cox et al., 2008). In the present study, diet was carefully supervised several days before the second game but only two meals before the first game. Thus, the possibility that the muscle CHO levels before the second game were higher than before the first game cannot be excluded. Noteworthy, blood glucose levels before and after the first and second games were comparable and similar amounts of a sports drink containing CHO was consumed during the games. Third, it has previously been reported that a second bout of exercise would cause a dampening of the inflammatory response (Pizza et al., 2001), the CK and delayed onset muscle soreness levels and blood oxidative stress markers (Nikolaidis et al., 2007), a concept called a ''repeated bout effect'' (McHugh, 2003). However, this phenomenon is reported to occur mostly in untrained subjects (Pizza et al., 2001; Nikolaidis et al., 2007). Because the participants in our study were well-trained soccer players, it is unlikely that the dampened cytokine response is related to the training status of the subjects. Additionally, in our study the immediate inflammatory cell count response and CK levels increased with the same magnitude following both games. In untrained subjects, the second exercise bout is accompanied by lower CK levels and inflammatory cell counts (Pizza et al., 2001; Nikolaidis et al., 2007). Fourth, the dampened cytokine response observed after the second game may be the result of a fast suppressive counter-regulation. Weinstock et al. (1997) suggested that the down-regulation of cytokine production a few hours after the exercise may be due to mediators such as cytokines, leukotrienes or prostaglandins (Weinstock et al., 1997). However, the second game was played 72 h after the first game. Whether the occurrence of the fast systemic counter-regulation following the first game might influence the cytokine response 72 h after the second game is unknown. Altogether, the mechanisms causing the dampened cytokine response after the second game and its implications for the recovery after soccer games need further investigations.

Interestingly, IL-12, IL-6, IL-8, MCP-1 and MIG increased following both games. IL-6 is part of the immune response but may also be modulated by metabolic factors during exercise (Cox et al., 2008). Moreover, it has been shown that IL-6, IL-8 and MCP-1 are produced locally in skeletal muscle during exercise (Pedersen et al., 2001; Warren et al., 2004; Åkerstrom et al., 2005) and are involved in muscle metabolism, angiogenesis, growth and regeneration (Warren et al., 2004; Nielsen & Pedersen, 2008). Also, MIG is proposed to act as a potent inhibitor of angiogenesis (Strieter et al., 2005), indicating its involvement in aspects other than the inflammatory process. Thus, the increases in IL-6, IL-8, MIG and MCP-1 following both games may reflect their involvement in different metabolic aspects in addition to inflammatory events. Unexpectedly, MIG increased following the second game despite the lack of an increase in INF-γ and INF-α. However, even though not significant, slight increases in INF-γ and INF-α were observed following the second game and it is possible that the non-significant increases in INF-γ and INF-α might have contributed to elevated MIG after the second game (Tables 1–3).

In conclusion, this study shows that an elite female soccer game leads to an immediate mobilization of immune cells and a robust, but transient, increase in the plasma concentration of both pro- and anti-inflammatory cytokines. Surprisingly, when a second game is played 72 h after the first game a dampened cytokine response occurs. Further investigations should aim to understand the mechanisms and the practical implications of the dampened cytokine response.

Perspectives

The finding that one elite female soccer game induces a well-orchestrated pro- and anti-inflammatory cytokine response does not support the use of anti-inflammatory drugs before or after one game in well-trained soccer players. It remains unknown whether a similar balanced pro- and anti-inflammatory cytokine response occurs even in untrained subjects. However, a dampening of the cytokine response, especially the anti-inflammatory cytokine response, occurs when two games are played within 72 h. At present, the implications of the dampened cytokine response in female players after the second game are unknown.

Key words: inflammation, intermittent exercise, active recovery, chemokines, training.

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