Effects of Caffeine on the Inflammatory Response Induced by a 15-km Run Competition

PEDRO TAULER1, SONIA MARTÍNEZ1, CARLOS MORENO1, MARTA MONJO2, PAU MARTÍNEZ1, and ANTONI AGUILÓ1

1Research Group on Evidence, Lifestyles and Health, Research Institute on Health Sciences (IUNICS), University of the Balearic Islands, Palma de Mallorca, SPAIN; and 2Group of Cell Therapy and Tissue Engineering, Research Institute on Health Sciences (IUNICS), University of the Balearic Islands, Palma de Mallorca, SPAIN

ABSTRACT

TAULER, P., S. MARTI´NEZ, C. MORENO, M. MONJO, P. MARTI ´NEZ, and A. AGUILO´ . Effects of Caffeine on the Inflammatory Response Induced by a 15-km Run Competition. Med. Sci. Sports Exerc., Vol. 45, No. 7, pp. 1269–1276, 2013. Purpose: The objective of this study is as follows: 1) to determine the effects of caffeine supplementation on the inflammatory response (IL-6 and IL-10 levels and leukocyte numbers) induced by a 15-km run competition and 2) to examine the effect of caffeine supplementation on the energetic metabolites as well as on the exercise-induced oxidative stress. Methods: A double-blinded study of supplementation with caffeine was performed. Athletes participating in the study (n = 33) completed a 15-km run competition. Before competition, athletes took 6 mg·kg⁻¹ body weight of caffeine (caffeine group, n = 17) or a placebo (placebo group, n = 16). Blood samples were taken before and after competition (immediately and after 2-h recovery). Leukocyte numbers were determined in blood. Concentrations of oxidative stress markers, antioxidants, interleukins (IL-6 and IL-10), caffeine, adrenaline, and energetic metabolites were measured in plasma or serum. Results: Caffeine supplementation induced higher increases in circulating total leukocytes and neutrophils, with significant differences between groups after recovery. Adrenaline, glucose, and lactate levels increased after exercise, with higher increases in the caffeine group. Exercise induced significant increases in IL-6 and IL-10 levels, with higher increases in the caffeine group. Caffeine supplementation induced higher increases in oxidative stress markers after the competition. Conclusion: Caffeine supplementation induced higher levels of IL-6 and IL-10 in response to exercise, enhancing the anti-inflammatory response. The caffeine-induced increase in adrenaline could be responsible for the higher increase in IL-6 levels, as well as for the increased lactate levels. Furthermore, caffeine seems to enhance oxidative stress induced by exercise. Key Words: CAFFEINE, ADRENALINE, IL-6, IL-10, LACTATE, OXIDATIVE STRESS

Numerous studies have reported increases in exercise performance after the ingestion of 3–6 mg·kg⁻¹ body mass of caffeine (13). In this sense, probably the most consistent observation is that caffeine can increase time to exhaustion during submaximal exercises lasting about 30–60 min (13). Nowadays, it is widely accepted that caffeine exerts mainly its effect via central fatigue mechanisms (13), by lowering the perception of effort during exercise and potentially allowing maintenance of performance when metabolic mechanisms are not limiting (8).

In addition to the ergogenic effects, caffeine influences the immunoenocrine response to exercise because of its influence, among others, on catecholamine levels and on the potent immunomodulator cyclic adenosine monophosphate (cAMP) (17). In fact, an increase in adrenaline, which mediates some of the changes included in the immune response to exercise (26), is a common finding when effects of caffeine supplementation are evaluated (4). On the other hand, it has been proposed that the increased levels of cAMP induced by caffeine results in immunomodulatory effects (9,17). In the last years, interleukin 6 (IL-6) has been proposed as the key factor in the inflammatory response to exercise, giving to that response anti-inflammatory properties (27,28). IL-6 is mainly produced and released by the contracting muscle, and it seems that this production increases in an exponential fashion when muscle glycogen levels become low (27). Regarding the inflammatory response to exercise, it has been suggested that IL-6 prevents increases in proinflammatory cytokines (tumor necrosis factor α, IL-1) and induces the production of anti-inflammatory cytokines such as IL-10 and IL-1ra (29).

Increased reactive oxygen species (ROS) formation could be involved in the regulation of IL-6 production (24,25). The possible links between ROS and IL-6 production include activation of the redox-sensitive transcription nuclear factor

Address for correspondence: Pedro Tauler, Ph.D., Department of Fundamental Biology and Health Sciences, University of the Balearic Islands, 07122-Palma de Mallorca, Spain; E-mail: pedro.tauler@uib.es.

Submitted for publication October 2012. Accepted for publication December 2012.

10133/13/4507-1269/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE©
Copyright © 2013 by the American College of Sports Medicine
DOI: 10.1249/MSS.0b013e3182857c8a
κB (11,24), the ROS induction of heat shock proteins, which in turn may activate IL-6 synthesis (24,25), and even the calcinerium–nuclear factor of activated T cells redox sensitive signaling pathway (24). It has been suggested that caffeine supplementation could increase oxidative stress induced by exercise (21,22). Several mechanisms related to caffeine-induced changes, such as higher lactate and adrenaline levels, could be involved. Higher proton release from increased lactic acid levels could involve higher ROS production because acidosis has been shown as a potent oxidative condition (14,19). Increased catecholamine concentrations could also contribute to enhanced oxidative stress because the catecholamine metabolism generates free radicals and other reactive species (3). Furthermore, the higher number of circulating neutrophils observed in caffeine-supplemented athletes after exercise, with similar or even increased cellular ROS production capability than the controls, could also contribute to oxidative stress (36). In addition, it has been reported that the hepatic metabolism of caffeine involves increased ROS production (35). However, only a few studies, with important limitations such as the lack of caffeine levels measurement, have examined the effects of caffeine supplementation on oxidative stress induced by exercise, showing controversial results after short exercise protocols (21,22).

Although caffeine has been suggested to be largely anti-inflammatory (17), only a few studies have evaluated the effects of caffeine supplementation on the changes in IL-6 levels induced by exercise, and no data regarding the changes of IL-10 has been found in the literature. Thus, the aim of the study was to determine the effects of caffeine supplementation on the inflammatory response (IL-6 and IL-10 levels and leukocyte numbers) induced by a 15-km run competition. The effect of caffeine supplementation on the energetic metabolites as well as on the exercise-induced oxidative stress was also evaluated. The main hypothesis was that caffeine could enhance the anti-inflammatory response to exercise, limiting the essential, but usually excessive, proinflammatory response to exercise.

MATERIALS AND METHODS

Subjects. Thirty-three voluntary male recreational athletes participated in this study. Table 1 shows the main characteristics of participants in the study. All the subjects were informed of the purpose and demands of the study before giving their written consent to participate. The protocol was in accordance with the Declaration of Helsinki for research on human subjects and was approved by the Ethical Committee of Clinical Investigation of the Balearic Islands. Participants in the study were instructed to avoid strenuous physical exercise and to abstain from taking caffeine for the 48 h previous to the competition. Subjects were enrolled after having fulfilled all inclusion criteria and presenting none of the exclusion criteria (determined by both questionnaire and interview). Subjects could be included if they were currently healthy, were engaged in at least 8 h of total moderate/high-intensity training or competition time per week, and were between 30 and 40 yr. Subjects representing one or more of the following criteria were excluded from participation: smoking, use of anti-inflammatory drugs within the previous 2 wk, and habitual consumption of antioxidant supplements for the last 3 months. In addition, subjects who never take caffeine were excluded from the study.

Experimental design. A double-blinded study of supplementation with caffeine was performed. At competition day, participants reported to the laboratory at 08:00 h, 3 h before the competition, after an overnight fast of 12 h. After 10 min of resting quietly, an initial (presupplement) blood sample was obtained from an antecubital forearm vein by venepuncture. Anthropometrical measurements were also determined. Two hours before the race, all volunteers consumed a specific breakfast comprising a sandwich (60 g bread, 25 g turkey or ham, 25 g cheese, and 20 g lettuce), 50 mL of decaffeinated coffee, 200 mL of semiskimmed milk, and water ad libitum.

An external researcher allocated study participants to the caffeine or placebo treatments. One hour before competition, athletes took 6 mg kg⁻¹ body weight of caffeine dissolved in 150 mL of “no-added sugar” yellow apple juice drink (caffeine group, n = 17) or a placebo, the same volume of the fruit juice (placebo group, n = 16). Both drinks were matched to be similar in taste and appearance. The apple juice content in vitamin C was lower than 2 mg per 100 mL. The exercise was a 15-km run competition. Heart rate was measured and recorded continuously using pulsometers (Suunto Team Pack Pro, Vantaa, Finland). During the race, only water was available for consumption at checkpoints along the racecourse. Subjects were not allowed to consume any food or beverages but water within 2 h after the race. Blood samples were also taken immediately after finishing the competition and 2 h after finishing the competition.

Two weeks before the competition day, subjects were asked to fill in a standard short-form International Physical Activity Questionnaire, thus providing quantitative information on training loads in MET-hours per week. Subjects were also asked to fill in a caffeine consumption questionnaire.

Table 1. Characteristics of participants and exercise performed.

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>39.6 ± 6.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.3 ± 7.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.9 ± 7.2</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>24.3 ± 2.0</td>
</tr>
<tr>
<td>Habitual caffeine intake (mg d⁻¹)</td>
<td>128 ± 34</td>
</tr>
<tr>
<td>Physical activity (MET·h wk⁻¹)</td>
<td>88.1 ± 56.8</td>
</tr>
<tr>
<td>VO₂peak (mL min⁻¹ kg⁻¹)</td>
<td>52.9 ± 4.4</td>
</tr>
<tr>
<td>Race time (min)</td>
<td>66.8 ± 5.6</td>
</tr>
<tr>
<td>Time per kilometer (min)</td>
<td>4.45 ± 0.39</td>
</tr>
<tr>
<td>Average speed (km h⁻¹)</td>
<td>13.7 ± 1.2</td>
</tr>
<tr>
<td>Resting HR (beats min⁻¹)</td>
<td>55.8 ± 11.0</td>
</tr>
<tr>
<td>HR max (beats min⁻¹)</td>
<td>178 ± 14</td>
</tr>
<tr>
<td>Average race HR (beats min⁻¹)</td>
<td>164 ± 7</td>
</tr>
<tr>
<td>Weight lost during exercise (kg)</td>
<td>0.4 1.9</td>
</tr>
<tr>
<td>Weight lost during exercise (%)</td>
<td>2.69 ± 0.61</td>
</tr>
</tbody>
</table>

The values are the mean ± SD (placebo group n = 17, supplemented group n = 16). *Indicates significant differences between groups (Student t-test for unpaired data). BMI, body mass index.
Blood sampling and measurements. Seated venous blood samples were collected in suitable vacutainers with EDTA as anticoagulant to obtain plasma and without additive to obtain serum. Within 30 min after blood collection, plasma and serum were obtained after centrifugation (15 min, 1000g, 4°C) of the blood samples obtained as indicated above. All the samples obtained were stored at −70°C until measurements were performed.

Cell numbers were quantified in an automatic flow cytometer analyser Techicon H2 (Bayer) VCS system. Hematocrit and hemoglobin were also determined for estimating plasma volume changes according to Dill and Costill (7). Concentrations of oxidative stress markers (malondialdehyde [MDA] and lipid hydroperoxides), antioxidants (vitamins A, E, and C, and uric acid), interleukins (IL-6 and IL-10), caffeine, adrenaline, and energetic metabolites (glucose, lactate, and free fatty acids) were measured in plasma or serum.

Plasma caffeine and adrenaline measurements. Caffeine was measured in plasma following an adaptation of the high-performance liquid chromatography (HPLC) method described by Blanchard et al. (5). After plasma deproteinization with methanol containing benzotriazol as internal standard, caffeine was determined at 273 nm. The mobile phase consisted of an 85/15 mixture of acetic acid buffer (pH 4.0, 10 mmol/L) and acetonitrile. The HPLC system was a Waters with a photodiode array detector, and the column was a Nova Pak, C18, 3.9 × 150 mm.

Adrenaline was measured in plasma using a commercially available high-sensitive and competitive enzyme-linked immunosorbent assay (LDN, Nordhorn, Germany) with a spectrophotometric microplate reader (PowerWave™; BioTek, Winooski, VT).

IL-6 and IL-10 concentrations. Concentrations of IL-6 and IL-10 were determined in plasma using commercially available ultrasensitive solid-phase sandwich enzyme-linked immunosorbent assays (Invitrogen, Carlsbad, CA) with a spectrophotometric microplate reader (PowerWave™; BioTek).

Glucose, lactate, and free fatty acid concentrations. Concentrations of glucose were measured in serum following standard procedures used in clinical biochemistry laboratory using an autoanalyzer Technicon DAX. Serum lactate and free fatty acid levels were measured using commercially available kits (BioVision Research Products, Mountain View, CA).

MDA and lipid hydroperoxides determinations. Lipid hydroperoxide was determined in serum following the method described by Gay and Gebicki (12). This method was based on the measure of the ferric–xylenol orange complex in a perchloric acid medium (PCA-FOX assay).

MDA was determined in plasma following the HPLC method described by Agarwal and Chase (1) with slight modifications. This method was based on the derivatization of MDA using 2-thiobarbituric acid, leading to the formation of the fluorescent MDA–thiobarbituric acid complex.

Plasma antioxidant vitamins determination. Vitamin A and α-tocopherol were determined in plasma after deproteinization with ethanol containing 0.2% butylated hydroxytoluene and extraction using n-hexane (33). The n-hexane extract was dried under a nitrogen current and redissolved in ethanol. An aliquot of the ethanol solution was injected in a Waters HPLC system with a photodiode array detector and a Nova Pak, C18, 3.9 × 150 mm column. The mobile phase consisted of 550/370/80 acetonitrile/tetrahydrofuran/H2O. α-tocopherol was determined at 290 nm and vitamin A at 330 nm.

Plasma ascorbate was determined by an HPLC method with electrochemical detection (33) after deproteinization with ortho-phosphoric acid. The mobile phase consisted of 0.05 M sodium phosphate, 0.05 M sodium acetate, 189 μM dodecyltrimethylammonium chloride, and 36.6 μM tetroctylammonium bromide in 25/75 methanol/H2O, pH 4.8. The HPLC system was a Waters with an electrochemical detector and a Nova Pak, C18, 3.9 × 300 mm column. The potential of the chromatographic detector was set at 0.7 V versus an Ag/AgCl reference electrode.

Statistical analysis. Statistical analysis was carried out using a statistical package for social sciences (IBM SPSS Statistics 19.0 for windows). Results are expressed as means ± SD, and P < 0.05 was considered statistically significant. All the data were tested for their normal distribution (Kolmogorov–Smirnov test). Student t-test for unpaired data was used to identify differences at baseline and in some characteristics of the exercise performed (e.g., HRmax and time). The effects of the supplementation on the changes induced by the competition were tested by a two-way ANOVA with supplementation (S) and the exercise (E) as ANOVA factors. When significant effects of caffeine supplementation or exercise were found, a one-way ANOVA with LSD post hoc test was used to determine the differences between the groups involved.

RESULTS

Table 1 shows the main characteristics of athletes belonging to both groups. No significant differences were found between participants belonging to the placebo and to the supplemented groups regarding age, anthropometrics, and physical activity performed during the two previous weeks. Furthermore, no differences between groups were found in the habitual caffeine intake. Taking into account the physical activity performed by athletes weeks before the competition, as well as the basal heart rate, they could be considered as well-trained recreational athletes. Furthermore, Table 1 shows parameters related to the exercise performed. The mean duration to complete the competition was similar in both groups, with athletes losing about 2 kg of weight during the race. The race average heart rate was significantly higher in the supplemented than that in the placebo group. However, no significant differences between groups were found in the race maximal heart rate, being the ones in the supplemented group slightly higher.
Table 2 shows plasma levels of caffeine and metabolites during the competition and the short recovery. ANOVA revealed a significant interaction between supplementation and exercise in caffeine, adrenaline, glucose, and lactate levels. Plasma caffeine was detected only in the supplemented group after supplementation, with similar high levels after the exercise and the recovery. Plasma adrenaline increased significantly in both groups after the competition, with a significantly higher increase in the supplemented group than that in the placebo one (177% vs 477%). Plasma glucose levels increased after the competition only in the supplemented group (48%). After the recovery, glucose levels in the supplemented group returned to basal levels. However, no significant differences between groups were observed in plasma free fatty acids levels, which increased significantly in both groups after the competition and remained high after the short recovery.

Table 3 shows the influence of exercise and caffeine supplementation on the changes in circulating white blood cell numbers. ANOVA showed a significant interaction between supplementation and exercise in total leukocyte and neutrophil numbers. Leukocyte number increased significantly after exercise, with similar values in both groups. However, caffeine supplementation induced higher increases in circulating total leukocytes after the short recovery period (106% in the placebo group and 157% in the supplemented group with respect to basal values). A similar pattern of change was observed in neutrophil number, with significant and similar increases in both groups after the exercise and higher increases in the caffeine group after recovery (220% in the placebo group and 327% in the supplemented one with respect to basal values). Lymphocyte number decreased significantly in both groups after the recovery, with no effect of caffeine.

Figure 1 shows the changes observed in IL-6 (A) and IL-10 (B) plasma concentrations in the placebo and the supplemented groups. For both interleukins, the statistical analysis revealed a significant interaction between caffeine supplementation and exercise. IL-6 increased significantly in both groups after the competition (placebo: 111%, supplemented: 271%), with levels in the supplemented group significantly higher than that in the placebo one. After recovery, IL-6 levels in the placebo group had returned to basal levels. However, in the supplemented group with supplementation and exercise (P < 0.05, two-way ANOVA).
group, IL-6 levels after recovery remained higher than basal ones. Regarding IL-10, significant increases were observed in both groups after the competition (placebo: 77%, supplemented: 161%), with higher postexercise levels in the supplemented group than that in the placebo one. In addition, after recovery, IL-10 plasma levels in the placebo group had returned to basal values, remaining higher in the supplemented group (112% with respect to basal values).

Table 4 shows the changes in plasma antioxidants and oxidative stress markers during competition and recovery in both groups. Slight but significant increases were observed in both groups after the competition in plasma vitamin C concentrations, which return to basal values after recovery. Uric acid increased after the competition, but the increase became significant just after the competition in the supplemented group and after the recovery in the placebo one. However, no effect of exercise and supplementation were observed on plasma vitamin A and E concentrations. On the other hand, significant effects of exercise and caffeine supplementation were observed in MDA and LOOH levels. LOOH and MDA increases after the competition were significant only in the supplemented group. However, no significant differences between groups were found in these postexercise values for both LOOH and MDA.

**DISCUSSION**

The main finding of the present study was that caffeine supplementation with 6 mg·kg\(^{-1}\) body weight induced higher increases in plasma concentrations of IL-6 and IL-10 after exercise. The fact that participants in the study could be considered as well-trained recreational athletes, together with the duration of the exercise, allowed us to establish a nonexhaustive exercise protocol, minimizing the effects of low muscle energetic availability and highlighting the effects of caffeine.

IL-6 is the first cytokine released into the circulation during exercise (27). Several studies have demonstrated that contracting skeletal muscle *per se* produces and releases IL-6 (27). The anti-inflammatory characteristics of the acute response induced by exercise were established, and it has been reported that IL-6 plays an important role modulating this response (26,27). In the present study, the competition induced modest changes in IL-6 plasma levels when compared with the increases obtained in similar but much longer competitions or exercise tests (23,30). IL-6 is involved in the regulation of the energetic metabolism during exercise, increasing fat oxidation and glucose uptake (27). In fact, it has been reported that muscle IL-6 release could be related to a low energy status in the muscles (27). Furthermore, IL-6 response to exercise was influenced by caffeine. We suggest that the higher increases after exercise in the caffeine group could be induced by adrenaline because, in agreement with previous studies (36,37), caffeine supplementation induced higher increases in adrenaline in response to exercise. It has been reported that muscle IL-6 release could be related to a low energy status in the muscles (27). However, the IL-6 response to exercise was influenced by caffeine. We suggest that the higher increases after exercise in the caffeine group could be induced by adrenaline because, in agreement with previous studies (36,37), caffeine supplementation induced higher increases in adrenaline in response to exercise. It has been reported that adrenaline plays a minor role in stimulating IL-6 during exercise because adrenaline infusion failed to mimic exercise-induced increase in IL-6, as a much lower increase in IL-6 was observed after infusion than after exercise (30). However, this study was developed using a strenuous exercise protocol that could involve an important decrease in glycogen stores. Authors of the study indicated that a decrease in the clearance of IL-6 could be responsible for the increase in plasma IL-6 induced by adrenaline (30), because when exercising and resting legs were under adrenaline exposure, only the exercising legs released detectable...
values are the mean ± SD (placebo group n = 17, supplemented group n = 16). * indicates significant differences versus before. S, significant effects of caffeine supplementation (S); E, significant effects of competition; S × E, significant interaction of supplementation and exercise (P < 0.05, two-way ANOVA).

TABLE 4. Changes in plasma antioxidant concentrations during competition and short recovery.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Recovery</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>578 ± 81</td>
<td>572 ± 88</td>
<td>591 ± 110</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>599 ± 121</td>
<td>587 ± 129</td>
<td>615 ± 134</td>
<td></td>
</tr>
<tr>
<td>α-tocopherol (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>8.48 ± 1.12</td>
<td>8.37 ± 1.29</td>
<td>8.70 ± 1.95</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>9.19 ± 1.81</td>
<td>8.68 ± 1.73</td>
<td>9.13 ± 1.68</td>
<td></td>
</tr>
<tr>
<td>Vitamin C (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>9.82 ± 1.98</td>
<td>11.43 ± 2.42</td>
<td>10.42 ± 2.73</td>
<td>E</td>
</tr>
<tr>
<td>Caffeine</td>
<td>9.97 ± 2.53</td>
<td>11.75 ± 2.88</td>
<td>10.49 ± 3.29</td>
<td></td>
</tr>
<tr>
<td>Uric acid (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>5.26 ± 0.69</td>
<td>5.74 ± 0.80</td>
<td>6.09 ± 0.71</td>
<td>E</td>
</tr>
<tr>
<td>Caffeine</td>
<td>5.18 ± 1.06</td>
<td>6.01 ± 0.94</td>
<td>6.44 ± 1.01</td>
<td></td>
</tr>
<tr>
<td>MDA (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>3.45 ± 1.40</td>
<td>3.87 ± 1.35</td>
<td>3.82 ± 1.41</td>
<td>E, S</td>
</tr>
<tr>
<td>Caffeine</td>
<td>3.59 ± 1.48</td>
<td>5.57 ± 1.98</td>
<td>4.07 ± 1.28</td>
<td></td>
</tr>
<tr>
<td>LOOH (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1.60 ± 0.38</td>
<td>1.72 ± 0.28</td>
<td>1.63 ± 0.17</td>
<td>E, S</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.56 ± 0.48</td>
<td>2.05 ± 0.42</td>
<td>1.71 ± 0.42</td>
<td></td>
</tr>
</tbody>
</table>

amounts of IL-6 (31). In this sense, it has been reported that the liver removes IL-6 from the circulation during exercise (10). On the other hand, it has been shown that adrenaline and noradrenaline, which could also increase in response to caffeine supplementation, induce, through α1-adrenergic receptor stimulation, a decrease in splanchnic blood flow due to vascular smooth muscle contraction and the consequent vasoconstriction (32). Thus, the decrease in the clearance of IL-6 in the supplemented group could be attributed to the adrenaline-induced reduced splanchnic blood flow. However, and as it was suggested, the elevated plasma IL-6 levels induced by adrenaline may also reflect an increase in IL-6 release from different tissues other than contracting muscle because almost any cell type may synthesize IL-6 upon adequate stimulation (2). In fact, higher IL-6 levels in the supplemented group could reflect an increase in IL-6 release from different tissues because of low energy status due to increased systemic energy expenditure induced by adrenaline (30).

In the present study, it has been found that IL-10 changes are also influenced by caffeine, with higher increases in the caffeine group. There are several mechanisms by which caffeine may increase IL-10 levels. One of them would involve IL-6, because it has been shown that IL-6 stimulates the production of IL-10 (29). However, this effect has been shown when increases in IL-6 are much higher than the one observed in the present study (29). This observation led us to think that the higher IL-10 increase in the caffeine group could be produced by cAMP because this immunomodulator induces IL-10 production (9), and it has been reported that cAMP increases after caffeine supplementation due to inhibition of cAMP-phosphodiesterase (17). In fact, one of the main limitations of the present study is that cAMP was not measured. It is generally believed that caffeine exerts most of its effects via antagonism of adenosine receptors (13,17). However, and taking into account the main adenosine receptors found in the immune cells involved in IL-10 production (16), caffeine, as an adenosine antagonist, would induce a decrease in cAMP levels, preventing the IL-10 increase. In this sense, it has been proposed that, regarding the anti-inflammatory effects of caffeine, the cAMP-phosphodiesterase inhibition may be a more important mechanism than adenosine receptor antagonism (17). Thus, results of the present study suggest that caffeine supplementation could play an anti-inflammatory role during exercise, enhancing, at least, the response of a well-known anti-inflammatory cytokine such as IL-10.

Results regarding the effect of caffeine on neutrophil and lymphocyte counts during and after exercise are controversial (4,36–38). It should be considered that in the present study, differences in neutrophil number between groups became significant after 2 h of recovery (delayed neutrophilia). Thus, the lack of concordance with previous studies could be explained taking into account the different recovery time followed: 2 h in the present study and 1 h in the previous ones (36–38). The delayed neutrophilia appears to be mediated by cortisol because it has been reported that exogenous cortisol infusion produces neutrophilia (26). Cortisol was not measured in this study. However, it seems that caffeine supplementation tends to induce higher levels of cortisol in response to exercise (36,37). Thus, differences between groups in the neutrophilia observed after exercise could be attributed to cortisol levels. These differences could also be attributed to higher levels of adrenaline or IL-6. However, previous studies failed to show that adrenaline infusion was able to mimic the exercise effect on neutrophils (30). On the other hand, it has been proposed that IL-6, after exercise, may play a role in the mobilization of neutrophils into the circulation (40). This suggestion was only based on significant correlations found between the neutrophil number and IL-6 levels after exercise (40), and similar correlations were found between the neutrophil number and hormones (e.g., cortisol and growth hormone) (40). Thus, it is possible that in the present study,
several factors such as the higher IL-6 increase, probably induced by adrenaline, and cortisol contribute to the higher neutrophilia in the caffeine group.

Caffeine supplementation has been reported to induce higher increases in glucose plasma levels after exercise (13). Although it has been suggested that caffeine induces lower rates of glucose uptake (34), it seems that this effect is limited to resting conditions and, maybe, the recovery after exercise (18). However, it was demonstrated that adrenaline increases glucose release to circulation by increasing hepatic glycogenolysis (6), which could be the main event inducing the higher plasma glucose levels in the caffeine group. Furthermore, higher IL-6 levels could also account for increased glucose levels because IL-6 has been shown to increase hepatic glucose output (27). On the other hand, results obtained in the present study confirm the finding that caffeine induces higher increases in lactate levels after exercise (13). Lactate accumulation in blood is dependent on the balance between lactate production by contracting muscle and lactate removal by liver, but also by other tissues such as exercising and nonexercising muscles. It has been suggested that adrenaline, through β-adrenergic receptor stimulation, plays an essential role in determining the result of this balance because it activates glycogenolysis and glycolysis (39), increasing lactate production, and also decreases lactate uptake by contracting muscles (15). However, all of these studies have been performed using adrenaline concentrations higher than typically observed during exercise. In fact, it should be indicated that some studies have not found any evidence regarding the effect of caffeine increasing lactate production, suggesting that increased lactate levels are produced because of decreased lactate clearance by resting muscles and liver (18). In this sense, the decreased lactate clearance by the liver could be produced because of the reduced splanchnic blood flow induced by adrenaline, as it has been discussed above for IL-6. However, and because it has been suggested that the liver could account for as much as 30% of lactate removal during exercise (20), more mechanisms could be involved.

The hypothesis that caffeine could increase oxidative stress induced by exercise was also tested. A few studies have suggested that caffeine supplementation enhances oxidative stress (21,22). In the present study, several parameters that could account for higher oxidative stress, as indicated previously, have been found to be significantly or slightly higher in the caffeine group after exercise such as lactate and adrenaline plasma levels and neutrophil circulating number. Thus, it is very difficult to attribute the differences to an only reason. However, the differences between groups regarding oxidative stress markers were not accompanied by differences in the antioxidant response to exercise. This hypothesis was also tested because it has been suggested that IL-6 production is regulated, at least in part, by redox sensitive factors (11,24,25). However, and in spite of some significant effects, the slight differences found in oxidative stress markers between groups did not allow explaining the differences in the IL-6 response to exercise in terms of the increased oxidative stress determined in the caffeine group.

In conclusion, although the effect of caffeine on IL-6 has been reported previously (38), to the best of our knowledge, this is the first study showing that caffeine supplementation induces higher levels of both IL-6 and IL-10 in response to exercise. However, more studies should be performed to evaluate the influence of caffeine on the whole inflammatory response to exercise. Because the energetic demands of the exercise performed did not compromise glycogen stores, the caffeine-induced increase in adrenaline could be the responsible for the higher increase in IL-6 in the supplemented group, as well as for the increased lactate levels. On the other hand, caffeine seems to enhance oxidative stress induced by exercise, although the possible mechanisms involved are presently unknown.

**REFERENCES**


We would like to thank all the participants in the study. Marta Monjo was supported by the Ministerio de Ciencia e Innovación del Gobierno de España (Ramón y Cajal contract).

No funding was received for this work.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

**RESPONSE TO EXERCISE AFTER CAFFEINE INTAKE**

**Medicine & Science in Sports & Exercise**


Copyright © 2013 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.