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ORIGINAL ARTICLE

Moderate swimming exercise and caffeine supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats

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Abstract The levels of circulatory inflammatory markers, including interleukin (IL) IL-1β, IL-6, tumor necrosis factor-α (TNF-α) and interferon (INF-γ), are known to increase associated to aging. Caffeine has been reported to produce many beneficial effects for health. Exercise is considered to be a safe medicine to attenuate inflammation and cellular senescence. The purpose of the present study was to investigate the effects of a moderate-intensity swimming exercise (3 % of body weight, 20 min per day, 4 weeks) and sub-chronic supplementation with caffeine (30 mg/kg, 4 weeks) on the serum cytokine levels in middle-aged (18 months) Wistar rats. The effects of swimming exercise and caffeine on oxidative stress in muscle and liver of middle-aged rats were also investigated. The two-way ANOVA of pro-inflammatory cytokine levels demonstrated a significant exercise x caffeine interaction for IL-1β ($F_{(1,\ 16)} = 9.5772$; $p = 0.0069$), IL-6 ($F_{(1,\ 16)} = 8.0463$; $p = 0.0119$) and INF-γ ($F_{(1,\ 16)} = 15.078$; $p = 0.0013$). The two-way ANOVA of TNF-α levels revealed a significant exercise × caffeine interaction ($F_{(1,\ 16)} = 9.6881$; $p = 0.00670$). Swimming exercise and caffeine supplementation increased the ratio of reduced glutathione/oxidized glutathione in the rat liver and gastrocnemius muscle. Hepatic and renal markers of damage were not modified. In conclusion, a moderate-intensity swimming exercise protocol and caffeine supplementation induced positive adaptations in modulating cytokine levels without causing oxidative stress in muscle and liver of middle-aged rats.

Keywords Middle-aged · Oxidative stress · C-reactive protein · Cytokines · Exercise · Caffeine

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Introduction

Cardiovascular disease, cancer and diabetes mellitus are chronic diseases, the largest cause of death in the world (Yach et al. 2004). Because of the aging and sedentary lifestyle the decline of immune function occurs, contributing to the increased incidence of these chronic diseases (Woods et al. 1999). Circulatory levels of inflammatory markers, including interleukin (IL) IL-1β, IL-6, C-reactive protein and tumor necrosis factor-α (TNF-α) are known to increase associated to aging, and thus they are frequently measured in research and diagnosis (Sjoholm and Nystrom 2006).

Exercise is considered to be a readily accessible and safe medicine to attenuate inflammation and cellular senescence (Werner et al. 2009; Peake et al. 2007). Although there are some discrepancies with respect to the effect of exercise on circulatory markers of inflammation, previous studies have

shown that aerobic exercise decreased production of the pro-inflammatory, atherogenic cytokines IL-1β, TNF-α, interferon gamma (INF-γ) and C-reactive protein levels and increased production of anti-inflammatory, athero protective cytokines, IL-10 (Goldhammer et al. 2005; Das 2004). By contrast, other studies have reported that exercise intervention had no such effects (Marcell et al. 2005). Moreover, exercise training results in an up-regulation of antioxidant defense mechanisms in various tissues (Calabrese and Baldwin 2001), presumably due to increased levels of oxidative stress that occurs during exercise (Deminice and Jordao 2012).

Caffeine is a member of the methylxanthine family and due to its presence in coffee, tea and medicinal products it is the most widely consumed psychoactive substance known in the world (Fredholm et al. 1999). Caffeine has been reported as a protective substance against cellular damage with beneficial antioxidant effects; probably due to the main metabolites of caffeine that are highly effective antioxidants (Chen and Whitford 1999). Epidemiologic studies have proven that intake of caffeine is associated with reduced levels of biomarkers of oxidative stress (Grucka-Mamczar et al. 2009; Ofluoglu et al. 2009). Moreover, the effect of caffeine on cytokine production has been demonstrated in vitro (Horrigan et al. 2004), but in vivo studies are scarce in the literature.

Based on the above considerations, the purpose of the present study was to investigate the effect of a moderate-intensity swimming exercise and supplementation with caffeine on the serum cytokine profiles in middle-age Wistar rats. The effect of swimming exercise and caffeine on oxidative stress in muscle and liver of middle-aged rats was also investigated.

Materials and methods

Animals

Middle-aged male Wistar Rats (18 months old, weighing 450–550 g) were obtained from a local breeding colony and were housed in cages, with free access to food and water. They were kept in a separate air-conditioned (22 ± 2 °C) room, on a 12-h light/12-h dark cycle, with lights on at 7:00 a.m. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.

Drugs

Caffeine was purchased from Sigma-Aldrich (Dorset, UK) and dissolved in warmed phosphate buffered saline. All other chemicals were obtained from analytical grade and standard commercial suppliers.

Caffeine supplementation

Middle-aged rats were separated into four groups: sedentary ($n = 8$), sedentary + caffeine ($n = 8$), exercise ($n = 8$) and exercise + caffeine ($n = 8$). Caffeine at a dose of 30 mg/kg (1 ml/kg) or phosphate buffered saline (vehicle) was administered by gavage 30 min before training, 5 days per week, for 4 weeks. The dose of 30 mg/kg, corresponding to the equivalent of 4–6 cups of coffee in humans, was chosen because it has previously shown to cause the maximal behavior effects in rodents (Fredholm et al. 1999).

Exercise training protocol

Animals from exercise and exercise + caffeine groups were submitted to the pre-training period (20 min/day, 5 days). After the swimming adaptation, rats were subjected to swimming training with a workload (3 % of body weight, 20 min per day for 4 weeks) (Ravi Kiran et al. 2004). Swimming training was performed in water at a temperature of 32 °C ± 1 between 19:00 and 21:00 p.m. Rats from sedentary and sedentary + caffeine groups were placed in the bottom of a separate tank with shallow water (5 cm) at 32 °C ± 1, without the workload (adaptation to the water). At the end of the exercise training, rats were towel dried and returned to their respective cages.

The individual body weight of rats was recorded two times a week throughout the experimental period. At the end of experimental period, adrenal and epididymal weights were recorded. Relative (to body weight) adrenal and epididymal weights were calculated.

After training protocol, rats were anesthetized with ketamine (90 mg/kg) and xylazine (5 mg/kg), for blood collection by heart puncture. Plasma was separated by centrifugation at 2,400×g for 10 min (hemolyzed plasma was discharged), and serum was also obtained. Samples of serum and plasma were stored at −20 °C for a 1-week period before biochemical analyses. Rats were killed by decapitation and gastrocnemius muscle and liver tissues were rapidly dissected, placed on ice and weighed.

Tissue preparation

Gastrocnemius muscle and liver tissues were immediately homogenized in cold 50-mM Tris–HCl buffer, pH 7.4 (1/10, weight/volume). Homogenate freshly prepared was centrifuged at 2,400×g for 10 min to yield a pellet that was discarded and a low-speed supernatant (S_1) was obtained. S_1 freshly prepared was used to determine glutathione

peroxidase and superoxide dismutase activities. Protein carbonyl was assayed using the fresh tissue homogenates (1/10, weight/volume) without centrifugation. Mitochondrial fraction was used to determine reactive species and GSH/GSSG levels.

Biochemical analyses

Cytokines

Serum interleukin (IL) IL-1β, IL-6, IL-10, tumor necrosis factor–α (TNF-α), interferon (INF-γ) and C-reactive protein determinations were performed using commercial ELISA kits as described by the manufacturer (eBIOSCIENCE, San Diego, USA). The results were expressed in pg/ml for IL-1β, IL-6, IL-10 and TNF-α, μg/ml for INF-γ and mg/l for C-reactive protein. The sensitivity of the assays for detection of cytokines was as stated in the manufacturer's brochures.

Hepatic and renal markers of damage

Plasma activities of alanine and aspartate aminotransferases (ALT and AST, respectively) and urea and creatinine levels were assayed spectrophotometrically using commercial kits (LABTEST, Diagnostica S.A., Minas Gerais, Brazil). The creatinine levels were expressed as mg/dl. Urea, AST and ALT activities were expressed as U/L.

Protein carbonyl

Carbonyl content was assayed by a method based on the reaction of protein carbonyls with dinitrophenylhydrazine (DNPH) forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm (Reznick and Packer 1994). Homogenates from gastrocnemius muscle and liver were diluted with Tris–HCl buffer, pH 7.4 in a proportion of 1:10. Aliquots of 940 μl of homogenate dilutions were incubated at 37 °C for 2 h. In two tubes, it was added 200 μl of 10 mM DNPH in 2.0 M HCl. In the third tube, only 200 μl of 2.0 M HCl solution (blank) was added. All tubes were incubated for 1 h at room temperature, in dark and shaken using a vortex mixer every 15 min. After that, 0.5 ml of denaturizing buffer (sodium phosphate buffer, pH 6.8, containing 3 % SDS), 1.5 ml of ethanol and 1.5 ml of hexane were added to all tubes. The tubes were shaken with a vortex mixer for 40 s and centrifuged for 15 min at 2,400×*g*. The pellet obtained was separated, washed two times with 1 ml of ethanol: ethyl acetate (1:1, volume/volume) and dried at room temperature for 2 min. The pellet was immediately dissolved in 1 ml of denaturizing buffer solution with mixing. Absorbance was measured at 370 nm. Results were expressed as carbonyl content (nmol carbonyl content/mg protein).

Superoxide dismutase

Superoxide dismutase (SOD) activity in S_1 was spectrophotometrically determined as described by (Misra and Fridovich 1972). This method is based on the capacity of SOD in inhibiting autoxidation of epinephrine. The color reaction was measured at 480 nm. S_1 from gastrocnemius muscle or liver was diluted 1:10 (v/v) for determination of SOD activity in the test day. Aliquots of supernatant were added in a 50 mM Na_2CO_3 buffer pH 10.2. The enzymatic reaction was started by adding of epinephrine. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50 % at 26 °C. The enzymatic activity was expressed as Units (U)/mg protein.

Glutathione peroxidase

Glutathione peroxidase (GPx) activity was spectrophotometrically determined by the method of (Wendel 1981), through the reduced glutathione (GSH)/reduced nicotinamide adenine dinucleotide phosphate (NADPH)/glutathione reductase (GR) system, by the dismutation of H_2O_2 at 340 nm. S_1 from gastrocnemius muscle or liver was added in GSH/NADPH/GR system and the enzymatic reaction was initiated by adding H_2O_2. In this assay, the enzyme activity is indirectly measured by means of NADPH decay. H_2O_2 is decomposed, generating oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by GR present in the assay media at the expenses of NADPH. The enzymatic activity was expressed as nmol NADPH/min/mg protein.

Mitochondrial isolation

Mitochondria were isolated as previously described (Bhattacharya et al. 1991), with some modifications. The liver and gastrocnemius muscle were rapidly removed and immersed in ice-cold isolation buffer I at 4 °C (100 mM sucrose, 10 mM EDTA, 100 mM Tris–HCl, 46 mM KCl, at pH 7.4). Aiming to promote homogenization and isolation of mitochondria samples of gastrocnemius, 1.2 mg/kg of trypsin was added to an isolation buffer I. After 30 min, the sample was neutralized with isolation buffer II. The tissue was then homogenized and the resulting suspension was centrifuged at 2,000×*g* for 7 min. After centrifugation, the supernatant was recentrifuged at 12,000×*g* for 12 min. The pellet was gently resuspended in isolation buffer II (100 mM sucrose, 10 mM EDTA, 100 mM Tris–HCl, 46 mM KCl and 0.5 % fatty-acid free bovine serum

albumin free of fatty acids, at pH 7.4) and centrifuged again at 12,000×*g* for 12 min. The supernatant was decanted, and the final pellet was gently washed and resuspended in isolation buffer III (270 mM mannitol, 70 mM sucrose, 20 mM Tris–HCl at pH 7.4). The entire mitochondrial preparation required less than 1 h to be isolated.

Reactive species

The mitochondrial generation of reactive species was determined by the fluorimetric method using the membrane permeable fluorescent dye, 2′,7′-dichlorofluorescein diacetate (DCHF-DA) assayed according to (Garcia-Ruiz et al. 1997). Briefly, the mitochondrial samples of liver and gastrocnemius muscle (150 µg of protein/ml) were incubated with isolation buffer III, and the respiratory substrates glutamate (5 mM) and succinate (5 mM). The reaction was started with the DCHF-DA (1 µM) addition, and the medium was kept at constant stirring during the assay period. The fluorescence analysis was performed at 488 nm for excitation and 525 nm for emission, with slit widths of 5 nm.

GSH/GSSG ratio

The mitochondrial GSH and GSSG levels were measured by the fluorimetric method (Hissin and Hilf 1976) with some modifications. Briefly, the mitochondrial preparations from gastrocnemius muscle and liver (500 µg protein/ml) were resuspended in sodium-phosphate buffer and phosphoric acid (H_3PO_4) 4.5 %, and were centrifuged at 100,000*g* for 30 min.

For GSH determination, 100 µl of the supernatant was added to 1.8 ml phosphate buffer and *o*-phthalaldehyde (1 mg/ml). After 15 min, the fluorescence was measured at 420 nm for emission and 350 nm for excitation, with slit widths of 3 nm.

For GSSG determination, 250 µl of the supernatant was added to *N*-ethylmaleimide (40 mM) and incubated at room temperature for 30 min. After the incubation 140 µl of the mixture was added to 1.76 ml NaOH (100 mM) solution and *o*-phthalaldehyde. GSH and GSSG levels were determined from comparisons with linear GSH and GSSG standard curves, respectively. Results were expressed as GSH/GSSG ratio.

Protein determination

Protein concentration was determined by the method previously described (Bradford 1976) using bovine serum albumin (1 mg/ml) as a standard.

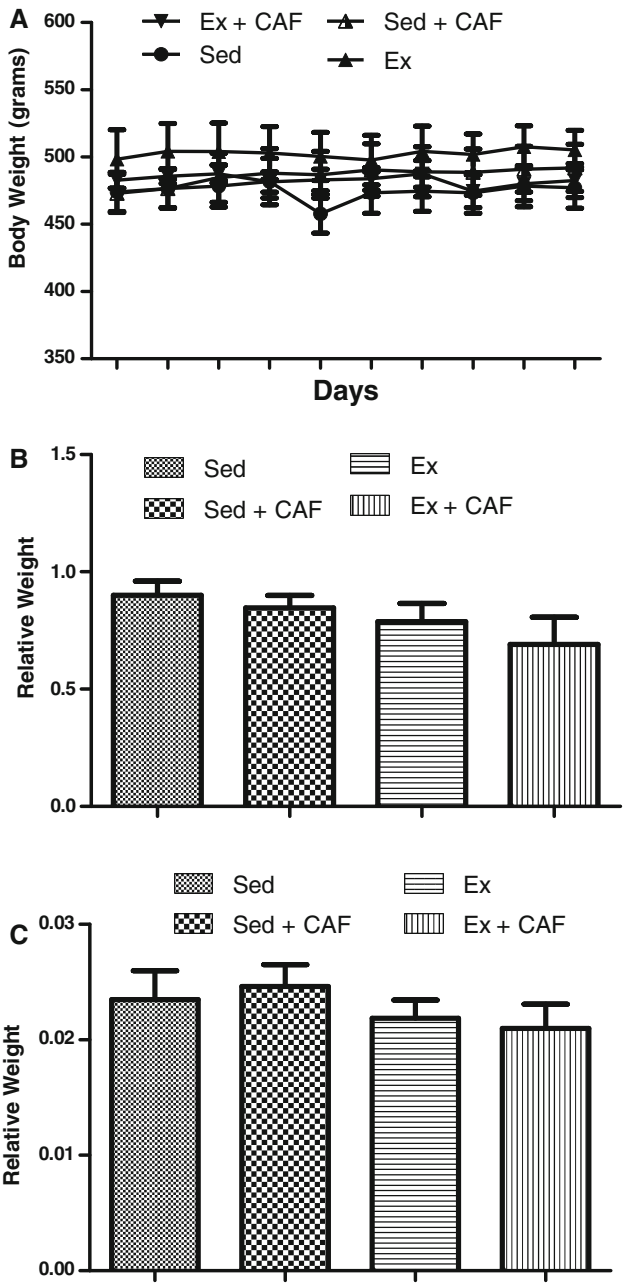

Fig. 1 Effect of caffeine supplementation (30 mg/kg, p.o., 5 days/week for 4 weeks) and swimming exercise on the body weight (**a**), relative epididymal weight (**b**) and relative adrenal weight (**c**) of middle-aged rats. *Sed* sedentary, *CAF* caffeine, *Ex* exercise

Statistical analysis

Data are expressed as mean ± standard error medium (SEM). Statistical analysis was performed using a Two-way ANOVA (interaction of exercise and caffeine) followed by the Duncan's test when appropriate. Values of $p < 0.05$ were considered statistically significant. Main effects are presented only when interaction was not significant.

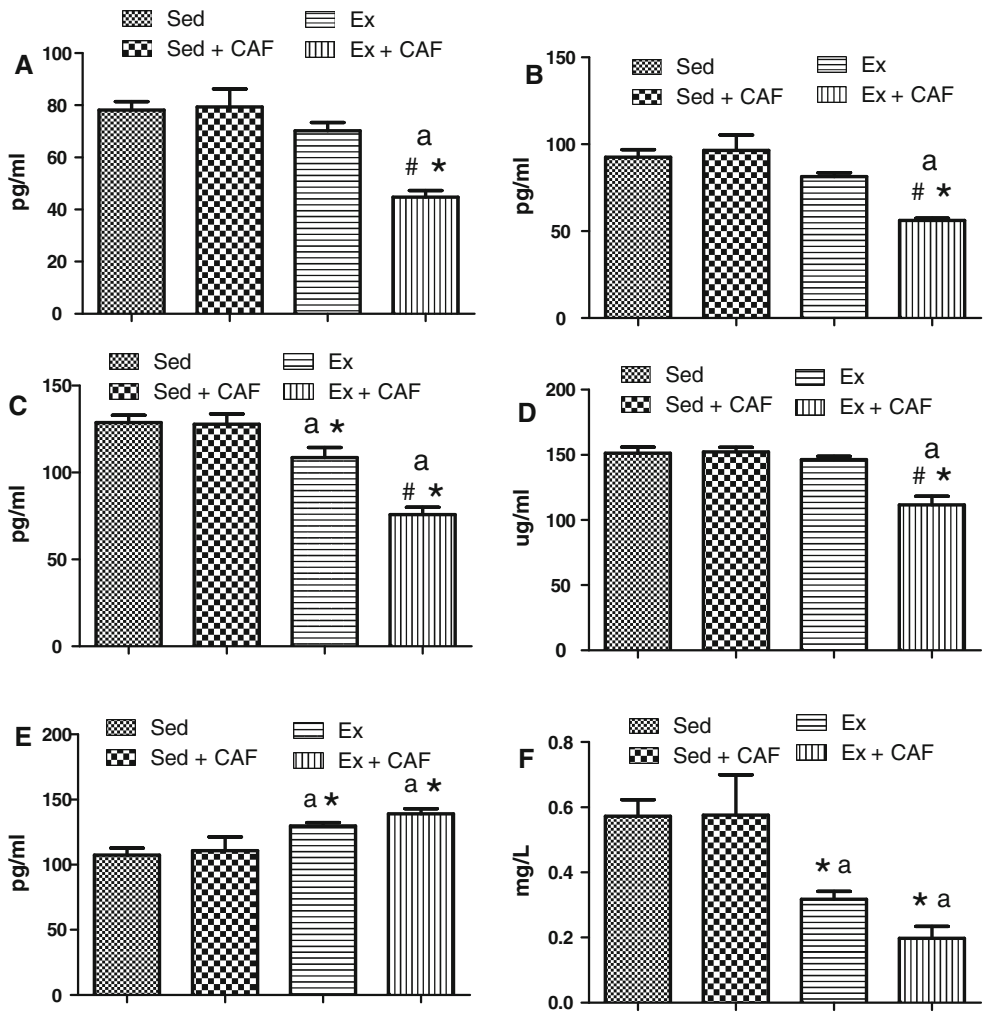

Fig. 2 Effects of caffeine supplementation (30 mg/kg, p.o.; 5 days/week for 4 weeks) and swimming exercise on inflammatory cytokines and C-reactive protein in the serum of middle-aged rats. Panel **a** IL-1β, **b** IL-6, **c** TNF-α, **d** INF-γ, **e** IL-10 and **f** C-Reactive Protein. Values are expressed as mean ± SEM ($n = 5$ rats per group). Data were analyzed by Two-way ANOVA followed by the Duncan's test. $^{*}p < 0.05$ as compared to sedentary rats; $^{\#}p < 0.05$ as compared to rats submitted to exercise, $^{a}p < 0.05$ as compared to sedentary rats treated with caffeine. *Sed* sedentary, *CAF* caffeine, *Ex* exercise

Results

There was no significant difference among groups in the body weight (Fig. 1a), relative epididymal weight (Fig. 1b) and adrenal weight (Fig. 1c) of middle-aged rats.

Middle-aged rats submitted to swimming training protocol and treated with caffeine showed a decrease in the levels of the pro-inflammatory markers. The two-way ANOVA of pro-inflammatory cytokines demonstrated a significant exercise x caffeine interaction for IL-1β ($F_{(1,\ 16)} = 9.5772$; $p = 0.0069$) (Fig. 2a), IL-6 ($F_{(1,\ 16)} = 8.0463$; $p = 0.0119$) (Fig. 2b) and INF-γ ($F_{(1,\ 16)} = 15.078$; $p = 0.0013$) (Fig. 2d).

The TNF-α levels were decreased in the exercise group and showed a further decrease in animals supplemented with caffeine and submitted to swimming exercise. The two-way ANOVA of TNF-α revealed a significant exercise x caffeine interaction ($F_{(1,\ 16)} = 9.6881$; $p = 0.00670$) (Fig. 2c).

The two-way ANOVA of data showed increased levels of IL-10 ($F_{(1,\ 16)} = 16.038$; $p = 0.0010$, Fig. 2e) and decreased levels of C-reactive protein ($F_{(1,\ 16)} = 20.236$; $p = 0.0003$, Fig. 2f) in animals of the exercise group and in those of the caffeine and swimming exercise group.

Parameters of oxidative stress are shown in Table 1. The ratio of GSH/GSSG levels was the unique parameter of oxidative stress investigated that showed significant differences. In gastrocnemius muscle, caffeine ($F_{(1,\ 14)} = 45.183$; $p = 0.00001$), exercise ($F_{(1,\ 14)} = 21.002$; $p = 0.00042$) and caffeine + exercise ($F_{(1,\ 14)} = 5.711$; $p = 0.03148$) groups increased the mitochondrial ratio of GSH/GSSG in relation to the sedentary group. The

Table 1 Effect of caffeine supplementation and swimming exercise on parameters of oxidative stress in gastrocnemius muscle and liver of middle-aged rats

	Groups	Carbonyl protein	Reactive species	GSH/GSSG	GPx	SOD
Gastrocnemius	Sed	2.76 ± 0.37	14.38 ± 1.63	4.55 ± 0.35	38.21 ± 5.48	17.61 ± 2.03
	CAF	3.22 ± 0.71	14.26 ± 1.74	6.48 ± 0.08*	37.08 ± 4.00	16.74 ± 1.52
	Ex	3.75 ± 0.50	14.98 ± 2.23	6.02 ± 0.19*	46.87 ± 6.99	13.51 ± 2.40
	Ex + CAF	3.27 ± 0.50	14.15 ± 1.45	6.94 ± 0.18*,#	41.27 ± 4.75	14.79 ± 2.20
Liver	Sed	1.11 ± 0.14	474.7 ± 42.78	3.44 ± 0.12	10.83 ± 1.74	22.19 ± 2.55
	CAF	1.52 ± 0.22	419.2 ± 39.70	4.78 ± 0.30*	9.18 ± 2.15	25.40 ± 1.86
	Ex	1.27 ± 0.11	412.1 ± 36.46	4.41 ± 0.25*	12.69 ± 3.36	20.20 ± 2.39
	Ex + CAF	1.11 ± 0.21	411.5 ± 67.76	4.71 ± 0.33*	11.20 ± 1.70	22.13 ± 0.91

Carbonyl protein (nmol carbonyl/mg protein), Reactive species (U.F. DCF-Oxid), GPx- glutathione peroxidase (nmol NADPH/mg protein/min), SOD superoxide dismutase (UI). Data were analyzed by Two-way ANOVA followed by the Duncan's test

Sed sedentary, *CAF* caffeine, *Ex* exercise, *CAF + Ex* caffeine + exercise

* $p < 0.05$ as compared to sedentary rats, # $p < 0.05$ as compared to rats submitted to exercise

Table 2 Effect of caffeine supplementation and swimming exercise on biochemical parameters middle-aged rats

Groups	Sed	CAF	Ex	Ex + CAF
AST	190.8 ± 27.59	185.8 ± 35.95	139.0 ± 16.04	131.0 ± 15.95
ALT	60.40 ± 3.20	43.80 ± 6.01	49.50 ± 5.04	45.67 ± 6.22
Urea	33.16 ± 5.79	28.24 ± 4.21	32.85 ± 8.36	35.20 ± 5.50
Creatinine	0.82 ± 0.08	0.76 ± 0.02	0.72 ± 0.02	0.90 ± 0.05

Sed sedentary, *CAF* caffeine, *Ex* exercise, *CAF + Ex* caffeine + exercise, *AST* aspartate transaminase (U/L), *ALT* alanine transaminase (U/L), urea (U/L) and creatinine (mg/dl)

caffeine + exercise group increased also the ratio of GSH/GSSG compared to the exercise group.

In the liver, all groups [caffeine ($F_{(1,\ 12)} = 70.675$; $p = 0.000002$), exercise ($F_{(1,\ 12)} = 62.495$; $p = 0.000004$) and caffeine + exercise ($F_{(1,\ 12)} = 6.670$; $p = 0.023977$)] increased the mitochondrial ratio of GSH/GSSG when compared to the sedentary group (Table 1).

The swimming training protocol and caffeine supplementation did not alter biochemical parameters in middle-aged rats (Table 2).

Discussion

In the current study, the effects of a moderate-intensity swimming training exercise and supplementation with caffeine on cytokine levels and oxidative stress in muscle and liver of middle-aged rats were demonstrated. The experimental protocol used in this study was effective in reducing pro-inflammatory cytokines and C-reactive protein as well as in increasing IL-10, an anti-inflammatory cytokine, in middle-aged rats. It is important to point out that the modulation of cytokine levels by swimming exercise and caffeine supplementation was independent of oxidative stress in muscle and liver of middle-aged rats.

Evidence has been found to associate exercise with the regulation of the inflammatory process (Werner et al. 2009; Lesniewski et al. 2011), which has been found to be an underlying contributor to many chronic diseases (Woods et al. 2009). In this context, greater levels of fitness are more associated with an anti-inflammatory profile than are lower degrees of physical fitness (Jankord and Jemiolo 2004). Ultramarathon and marathon runners have been reported to have lower C-reactive protein levels compared with sedentary controls (Mattusch et al. 2000; Tomaszewski et al. 2003). Other studies have also reported inverse associations between fitness status and C-reactive protein in humans (Borodulin et al. 2006; Aronson et al. 2004; Church et al. 2002). Regarding caffeine, its biochemical and physiological effects including anti-inflammatory (Back et al. 2006; Paur et al. 2010) have been studied.

The reduction of inflammatory cytokine levels was clearly demonstrated in this experimental protocol, but the isolated effect of caffeine or exercise was discrete or not apparent. In fact, this training protocol reduced the levels of TNF-α and C-reactive protein and increased the levels of IL-10, while the supplementation to a relatively high dose of caffeine was not effective in modulating the levels of cytokines in serum of middle-aged rats. Taken these data

collectively, it is possible to assume that exercise and caffeine are working together in modulating inflammatory cytokines.

Moreover, the beneficial effects of a moderate-intensity swimming training exercise and caffeine supplementation in reducing serum levels of pro-inflammatory cytokines and C-reactive protein in middle-aged rats were independent of the loss of body weight. The effect of loss in the body fat content has been implicated in the reduction of serum IL-6 and TNF-α and as a consequence, the reduction in C-reactive protein (Mathieu et al. 2009), but exercise-induced C-reactive protein reduction was not associated with weight loss (Okita et al. 2004).

One possible explanation for the modulation of inflammatory cytokines by exercise could be centered in the increased mononuclear cell production of IL-10 and other anti-inflammatory cytokines (Pretolani 1999) while decreased production of pro-inflammatory cytokines (Smith et al. 1999).

TNF-α and IL-6 are the main inducers of hepatic production of acute phase proteins, including C-reactive protein. In the swimming exercise and caffeine protocol, the levels of TNF-α and IL-6 were reduced, which could account to the reduction in C-reactive protein levels. Moreover, the effects of a moderate-intensity swimming exercise protocol and caffeine supplementation by maintaining the healthy levels of IL-10 appear to provide a protective environment for tissue in middle-aged rats. In this context, the mechanisms by which a moderate-intensity exercise or caffeine exerts beneficial effects on cytokines have been reported (Pedersen and Hoffman-Goetz 2000; Lira et al. 2009; Petersen and Pedersen 2005; Lv et al. 2010; Yun et al. 2008).

Exercise-induced increased production of reactive oxygen species can be beneficial by evoking specific adaptations, such as increased antioxidant/oxidative damage repairing enzyme activity, increased resistance to oxidative stress and lower levels of oxidative damage (Calabrese and Baldwin 2001). In this context, it has been postulated that the concept of hormesis can be applied to exercise-induced gene expression of antioxidant enzymes (Ji et al. 2006).

Cells have evolved highly complex enzymatic and nonenzymatic antioxidant systems which work synergistically, and in combination with each other, to protect the body against free radical-induced damage (Lee et al. 2011; Golbidi and Laher 2010). Glutathione is the most abundant low molecular weight tripeptide thiol in living cells and exists in reduced (GSH) and oxidized (GSSG) states (Rossowska and Nakamoto 1994). The benefits of a moderate-intensity swimming exercise protocol and caffeine supplementation were also demonstrated in this study by the absence of reactive species and carbonyl protein formation and by the increase of GSH/GSSG ratio, a marker of the oxidative stress, in the gastrocnemius muscle of middle-aged rats. It is worth mentioning that, excepting the GSH/GSSG ratio, none of parameters of oxidative stress was altered in the livers and muscle of middle-aged rats.

There is evidence that higher coffee/caffeine consumption reduces the elevation of serum AST and ALT, which are markers of liver injury (Cadden et al. 2007). In the present study, a moderate swimming exercise protocol and caffeine supplementation either alone or associated did not alter body adiposity, plasma levels of urea and creatinine as well as AST and ALT activities.

Regarding the relevance of the results obtained in this study for humans, we acknowledge that differences between animals and humans make extrapolation of animal data very difficult. Tentatively, one can affirm that caffeine is one of the most widely used pharmacologically active drugs in the world and is found in different products, energy beverages, dietary supplements and over-the-counter medications. It has been estimated that worldwide caffeine consumption is approximately 76 mg/day per person; in the United States and other developed countries, the average consumption rate exceeds 230 mg/day (Magkos and Kavouras 2005). Moreover, this drug is commonly used within sport and exercise settings for its performance-enhancing effects (Graham 2001; Arciero and Ormsbee 2009). The benefits of high dose of caffeine before exercise in humans have been poorly explored (Spriet et al. 1992).

Conclusion

In conclusion, a moderate-intensity swimming exercise protocol and caffeine supplementation induced positive adaptations in modulating cytokine levels, reducing pro-inflammatory cytokines and increasing IL-10, an anti-inflammatory cytokine, without causing oxidative stress in muscle and liver of middle-aged rats.

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Conflict of interest The authors declare they have no conflicts of interest to disclose.

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