Life Sciences 96 (2014) 40–45

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Caffeine supplementation modulates oxidative stress markers in the liver of trained rats

Rômulo Pillon Barcelos [a], Mauren Assis Souza [a,b], Guilherme Pires Amaral [a], Silvio Terra Stefanello [a], Guilherme Bresciani [a,b], Michele Rechia Fighera [b,c], Félix Alexandre Antunes Soares [a,b,*], Nilda Vargas Barbosa [a]

[a] Departamento de Química, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[b] Laboratório de Bioquímica do Exercício (BioEx), Centro de Educação Física e Desportos, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[c] Departamento de Neuropsiquiatria, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 1 October 2013
Accepted 3 December 2013

Keywords:
Caffeine
Exercise training
Liver
Oxidative stress
Redox status modulation

ABSTRACT

Aims: Caffeine has been widely used in sports competitions due to its ergogenic effects. Most of the studies regarding caffeine and exercise have focused on muscle and plasma adaptations, while the impact on the liver is scarcely described. The aim is to analyze the effects of caffeine and exercise training on oxidative stress markers and injury-related parameters in the liver.
Main methods: Rats were divided into sedentary/saline, sedentary/caffeine, exercise/saline, and exercise/caffeine groups. Exercise groups underwent 4 weeks of swimming training, and caffeine (6 mg/kg, p.o.) was supplemented throughout the training protocol. Injury-related liver parameters were assessed in plasma, while redox status and oxidative stress markers were measured on liver homogenates.
Key findings: Exercise training increased muscle citrate synthase activity in the muscle, while in caffeine decreased its activity in both sedentary and trained rats. Aspartate transaminase levels were increased after training, and caffeine intake suppressed this elevation ($p < 0.05$). Caffeine also diminished alanine transaminase levels in both sedentary and exercised rats ($p < 0.05$). Exercise training induced a significant increase on the activity of the enzymes superoxide dismutase and glutathione peroxidase, as an increase on thiobarbituric acid-reactive substances levels was also reached ($p < 0.05$); caffeine intake blunted these alterations. Caffeine intake also suppressed liver catalase activity in both sedentary and exercise groups ($p < 0.05$).
Significance: Our data suggest that caffeine modified the hepatic responses associated to exercise-induced oxidative stress without affecting the performance, exerting different actions according to the tissue. However, further studies are needed to better understand caffeine's role on liver under exercise training.

Introduction

Caffeine (1,3,7-trimethylxanthine) is a xanthine alkaloid compound present in many commercial beverages and medicines that acts as a potent stimulant of the central nervous system (CNS) (Tunnicliffe et al., 2008). In addition, caffeine incites skeletal muscle metabolism and therefore it has been widely used in athletic competitions due to its ergogenic effects (Kalmar and Cafarelli, 2004; Tarnopolsky, 2008; Goldstein et al., 2010). Its widespread use is allowed by the World Anti-Doping Agency's despite evidence-based data that documents its physiological and performance-enhancing effects (Tarnopolsky, 2010). In this line, it is well known that caffeine may affect substrate utilization during exercise (Tunnicliffe et al., 2008; Goldstein et al., 2010; Yang et al., 2009; Davis et al., 2003). Caffeine increases fatty acid mobilization during exercise thus decreasing glycogen reliance during performance (Ivy et al., 1979; Erickson et al., 1987; Spriet et al., 1992). These effects have been broadly linked to improvements on aerobic exercise performance due to enhanced twitch strength of both skeletal and cardiac muscles, which result in delay fatigue onset (Goldstein et al., 2010; Tarnopolsky and Cupido, 2000; Simmonds et al., 2010).

On the same line, it is well known that regular exercise training plays a protective role against lifestyle-related diseases across health status and quality of life improvements (Radak et al., 2004, 2005a,b). Accordingly, it has been stated that regular exercise training may increase the resistance to various stressors via hormesis (Radak et al., 2005a). The molecular events involved in this regulation may be linked to redox status homeostasis, an oxidative stress-related adaptive response (Radak et al., 2008; Jackson, 2008; Gomez-Cabrera et al., 2008). In fact, exercise training incites regular adaptations to the continuous presence of small stimuli such as mild amounts of reactive oxygen species (ROS). In this case, the regular stimuli can trigger the expression of antioxidant enzymes and modulates other oxidative stress markers (Radak et al., 2005a; Jackson, 2008; Gomez-Cabrera et al., 2008; Ji, 1993).

* Corresponding author at: Departamento de Química, CCNE, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. Tel.: +55 55 3220 9558.
E-mail address: felix@ufsm.br (F.A.A. Soares).

0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.12.002

Although growing evidence shows the beneficial effects of caffeine intake on skeletal muscle performance during exercise training, the role of caffeine in other tissues is scarcely described. Specifically, the influence of caffeine on the oxidative responses elicited by exercise training has been mostly limited to the skeletal muscle, brain and plasma samples. Therefore, considering the remarkable metabolic role of the liver during exercise, this study aimed to analyze the isolated and/or combined effects of caffeine supplementation and exercise training on oxidative stress and tissue injury-related markers.

Materials and methods

Ethical approval

The experimental assays were conducted in accordance to national and international legislations (Brazilian College of Animal Experimentation (COBEA) and the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals-PHS Policy). The study protocol was also approved by the Ethics Committee for Animal Research of the Universidade Federal de Santa Maria (UFSM, permit number 115/2010) before experimental set beginning.

Animals and reagents

Male Wistar rats (180–250 g) were obtained from our own breeding colony and kept in plastic boxes containing a maximum of five animals per cage. After, cages were placed in controlled environment conditions (12:12 h light–dark cycle, with onset of light phase at 7:00, 25 ± 1 °C, 55% relative humidity) with food (Guabi, Santa Maria, Brazil) and water ad libitum. Assay reagents were purchased from Sigma (St. Louis, MO, USA), and the other chemicals used in this study were of analytical grade and obtained from standard commercial suppliers.

Study design

The animals were randomly divided into four groups (n = 8): sedentary-saline (SED-SAL), sedentary-caffeine (SED-CAF), exercise-saline (EXE-SAL) and exercise-caffeine (EXE-CAF). The exercise and sedentary groups received caffeine (6 mg/kg in saline) or its vehicle by intragastric gavage (p.o.) through the experimental period.

Training protocol

For exercise training, animals were weighed and randomly assigned to the aforementioned groups. The tank used in this study was 80 cm in length, 50 cm in width, and 90 cm in depth, and the swimming training was performed in water temperature of 31 ± 1 °C (70 cm depth) between 10 and 12 h am. The training session consisted of 50 min per day and 5 days per week (Song et al., 1998). The EXE groups performed swimming training with a 5% body weight overload attached to the back to improve endurance (Lima et al., 2013; Gobatto et al., 2001). The SED groups were placed in a separate but similar tank with shallow water (5 cm) at the same temperature for 30 min, 5 days a week without the back overload. Caffeine supplementation (6 mg/kg) was administered daily throughout the training protocol (Fredholm et al., 1999).

Tissue sampling, and organs weighting

At the end of the exercise training protocol, the rats were euthanized and, liver, adrenal gland, soleus and gastrocnemius muscles were removed and weighted. A single ratio between organ and total body weights was calculated to express this data. Samples of liver and gastrocnemius muscle were quickly removed, placed on ice, and homogenized within 10 min in 10 volumes of cold Tris 10 mM (pH 7.4). Liver and muscle homogenates were centrifuged at 4000 ×*g* at 4 °C for 10 min to yield the low-speed supernatant fraction that was used for different biochemical assays in all trials. Blood samples were collected and centrifuged 1500 ×*g* for 10 min for plasma isolation in order to perform biochemical analysis.

Plasma assays

Biochemical parameters

Plasma levels of creatinine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), triglycerides (TG), total cholesterol (TC), uric acid (UA), high density lipoprotein (HDL) and urea (UR) were estimated by standard commercially biological kits (Labtest, Lagoa Santa, Brazil).

Estimation of DNA damage

The cell death indicated by the presence of double strand DNA in the plasma (dsDNA) was measured using the PicoGreen® fluorescent assay (Ahn et al., 1996). The assay was performed according to protocol supplied by the manufacturer (Quant ItTM, Invitrogen, Brazil). The fluorescence measurements were recorded on a fluorimeter, and fluorescence emission of PicoGreen® alone (blank) and PicoGreen® with DNA were recorded at 520 nm using an excitation wavelength of 480 nm at room temperature (25 °C). The results were expressed as % of control.

Citrate synthase (CS) activity

Citrate synthase activity was determined spectrophotometrically in mixed gastrocnemius muscle and liver according to the method previously described (Srere, 1968). The enzyme activity was measured in homogenates and the amount of the complex resulting from acetyl-CoA and oxaloacetate was determined at 412 nm and 25 °C. The CS activity was expressed as percentage of control.

Liver homogenate assays

Catalase (CAT) activity

The CAT enzyme activity was determined according to the method proposed by Aebi (1984). The kinetic analysis of CAT was started after H_2O_2 addition and the color reaction was measured at 240 nm. Data were corrected by the protein content and expressed as percentage of control.

Superoxide dismutase (SOD) activity

The SOD enzyme activity was determined according to the method proposed by Misra and Fridovich (1972). The kinetic analysis of SOD was started after adrenaline addition, and the color reaction was measured at 480 nm. Data were corrected by the protein content and expressed as percentage of control.

Glutathione peroxidase (GPx) activity

The GPx activity was determined spectrophotometrically at 340 nm by NADPH consumption for 2 min at 30 °C (Flohé and Günzler, 1984). The reaction was initiated by adding the H_2O_2 to a final concentration of 0.4 mM. The GPx activity was determined using the molar extinction coefficient 6220 M^{-1} cm^{-1} and expressed as percentage of control.

Glutathione reductase (GR) activity

For GR activity determination, the measurements were made at 340 nm and initiated with addition of 20 mM GSSG, at 30 °C for 2 min (Carlberg and Mannervik, 1985). The GR activity was determined using the molar extinction coefficient 6220 M^{-1} cm^{-1}, corrected by the protein content, and expressed as percentage of control.

Fluorimetric assay of reduced (GSH) and oxidized glutathione (GSSG)

For measurement of GSH and GSSG levels, the method previously described by Hissin and Hilf (1976) was used (Hissin and Hilf, 1976).