Toxicology Reports 5 (2018) 598–607

Contents lists available at ScienceDirect

Toxicology Reports

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

Capsaicinoids improve consequences of physical activity

Kazim Sahin[a,*], Cemal Orhan[a], Mehmet Tuzcu[b], Nurhan Sahin[a], Fusun Erten[b], Vijaya Juturu[c]

[a] Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, Elazig, Turkey
[b] Division of Biology, Faculty of Science, Firat University, Elazig, Turkey
[c] Research and Development, Clinical Affairs, OmniActive Health Technologies Inc., Morristown, NJ, USA

ARTICLE INFO

Keywords:
Exercise
Capsaicinoid
PPAR-γ
Nrf2
SREBP-1c

ABSTRACT

The purpose of this study was to investigate the effects of capsaicinoids (CAPs) on lipid metabolism, inflammation, antioxidant status and the changes in gene products involved in these metabolic functions in exercised rats. A total of 28 male Wistar albino rats were randomly divided into four groups (n = 7) (i) No exercise and no CAPs, (ii) No exercise + CAPs (iii) Regular exercise, (iv) Regular exercise + CAPs. Rats were administered as 0.2 mg capsaicinoids from 10 mg/kg BW/day Capsimax® daily for 8 weeks. A significant decrease in lactate and malondialdehyde (MDA) levels and increase in activities of antioxidant enzymes were observed in the combination of regular exercise and CAPs group ($P < 0.0001$). Regular exercise + CAPs treated rats had greater nuclear factor-E2-related factor-2 (Nrf2) and heme oxygenase-1 (HO-1) levels in muscle than regular exercise and no exercise rats ($P < 0.001$). Nevertheless, regular exercise + CAPs treated had lower nuclear factor kappa B (NF-κB) and IL-10 levels in muscle than regular exercise and control rats ($P < 0.001$). Muscle sterol regulatory element-binding protein 1c (SREBP-1c), liver X receptors (LXR), ATP citrate lyase (ACLY) and fatty acid synthase (FAS) levels in the regular exercise + CAPs group were lower than all groups ($P < 0.05$). However, muscle PPAR-γ level was higher in the regular exercise and CAPs alone than the no exercise rats. These results suggest CAPs with regular exercise may enhance lipid metabolism by regulation of gene products involved in lipid and antioxidant metabolism including SREBP-1c, PPAR-γ, and Nrf2 pathways in rats.

1. Introduction

Regular physical exercise has many useful benefits on human health such as prevention of chronic diseases. In recent years, the frequency of chronic diseases has been increasing in the world including disorders of carbohydrate and lipid metabolism [1]. There are many studies showing that regular exercise has positive effects on lipid metabolism as well [1–3]. These studies have shown that regular exercise improves insulin sensitivity, decreases risk factors for metabolic syndrome [4], improves beneficial factors related to muscle metabolism [5], increases brain volume and improves cognitive function [6] and reduces oxidative stress [7,8]. Additionally, physical exercise improves muscle mass and muscle function by increasing muscle protein synthesis and a stimulation of mitochondrial biogenesis [9,10]. However, in some studies conducted in humans and animals exposed to treadmill running at 20 m/min on a 15% incline for 60 min/day (5 days/wk) showed that high-intensity exercise causes oxidative damage to biomolecules, and this process is related to the increased metabolism that occurs when the body is exposed to intense exercise [11–13]. In addition, Spanidis et al., [13] reported that static oxidation-reduction potential marker and total antioxidant capacity was improved in athletes, whereas, thiobarbituric acid reactive substances (TBARS), protein carbonyls and the total antioxidant capacity (TAC) showed obvious variations in the athletes, suggesting that the optimum approach with which to counteract including antioxidant supplementation.

In recent years, carbohydrate and lipid metabolism disorders resulting from inadequate exercise or unbalanced nutrition could induce hyperlipidemia, diabetes and other lipid metabolism disorders by regulating signal pathways including transcription factors [the sterol regulatory element-binding protein 1 (SREBP-1) and liver X receptors

Abbreviations: ACLY, ATP-citrate lyase; ACS, acetyl-CoA synthetase; AMPK, phosphorylated AMP-activated protein kinase; ARE, antioxidant response element; CAPs, capsaicinoids; FAS, fatty acid synthase; GSH-Px, glutathione peroxidase; HO-1, heme-oxygenase 1; IL-10, interleukin-10; LXR-s, liver X receptor-s; MMP-9, matrix metalloproteinase-9; MDA, malondialdehyde; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PGC-la, peroxisomal proliferator activator receptor c coactivator; PPAR-γ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SOD, superoxide dismutase; SREBP-1c, sterol regulatory element-binding protein1c; TC, total serum cholesterol; Tfam, mitochondrial transcription factor A; TG, triglyceride; TRPV1, transient receptor potential vanilloid subtype 1; TNF-α, tumor necrosis factor-α

* Corresponding author: Veterinary Faculty, Firat University, 23119, Elazig, Turkey.
E-mail addresses: ksahin@firat.edu.tr (K. Sahin), corhan@firat.edu.tr (C. Orhan), mtuzcu@firat.edu.tr (M. Tuzcu), nsahin@firat.edu.tr (N. Sahin), fusun_78@yahoo.com (F. Erten), v.juturu@omniactives.com (V. Juturu).

https://doi.org/10.1016/j.toxrep.2018.05.001
Received 14 February 2018; Received in revised form 16 April 2018; Accepted 13 May 2018
Available online 15 May 2018

(LXR) and several enzymes including ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)], antioxidant metabolism such as nuclear factor-E2-related factor-2 (Nrf2) and inflammation e.g. nuclear factor kappa B (NF-κB) [2,14]. Nrf2 is the key regulator of antioxidant signaling, which plays an important role in the regulation of blood sugar and insulin sensitivity [15]. SREBP-1 may control the ectopic accumulation of fat and may activate the target gene FAS, an important enzyme that regulates the levels of fatty acid synthesis [14]. LXRs regulate fatty acid and cholesterol homeostasis and are expressed mainly in the liver and other tissues involved in lipid metabolism [16]. AMP-activated kinase (AMPK), a key regulator of energy metabolic homeostasis, can play an important role in regulating the synthesis of fatty acids, cholesterol, glucose, and hepatic gluconeogenesis [17].

Currently, the use of naturally occurring antioxidants such as coenzyme Q10 [18,19], curcumin [8], quercetin [20] and resveratrol [21] improve performance and exercise capacity during physical exercises. Similarly, polyphenolic extracts with antioxidant properties are also effective to improve antioxidant capacity in endothelial and muscle cells [22]. These antioxidants have a bioenergetics and antioxidant system reinforcement role, induce skeletal muscle and brain mitochondrial biogenesis. Meanwhile, capsaicinoids (CAPs), which have high anti-inflammatory and antioxidant properties and are widely used in traditional medicine, but which are lacking in research on sports supplements. Capsaicinoids are the major pungent, naturally occurring active compounds in *Capsicum* fruits such as hot chili peppers (genus *Capsicum*), with the most abundant forms being capsaicin, dihydrocapsaicin, and nordihydrocapsaicin [23,24]. Capsaicinoids have many biological and physiological activities including anti-inflammatory [25], antioxidant [26], anticancer [27], thermogenesis [28] and lipolysis enhancer [24], hormone function improver [29] and blood glucose regulation [30]. It has also been reported that consumption of food containing capsaicinoids is associated with a lower incidence of lipid metabolism disorders [31].

There are also thermogenic and appetitive effects of capsaicin [32,33]. Janssens et al. [33] reported that consumption of 2.56 mg capsaicin per meal promoted fat oxidation in negative energy balance and did not increase blood pressure significantly. In addition, it was reported that capsiate activates transient receptor potential vanilloid subtype 1 (TRPV1) receptors in the gut but not in the oral cavity [32]. Although the effects of CAPs have been demonstrated in vitro and in vivo studies, the effects of CAPs on the major transcriptional regulator of fatty acid (SREBP-1) and some key transcription factors (NF-κB and Nrf2) have not been fully investigated in exercise-treated rats. Therefore, this study was designed to investigate the effects of exercise and CAPs on lipid metabolism, inflammation and oxidative stress in regularly trained rats. Furthermore, its mechanisms of attenuation of metabolic profiles, inflammation, and oxidative stress were elucidated by evaluating NF-κB and Nrf2 pathways as well as SREBP-1, LXR, ACL, ACC, FAS, and PPAR-γ.

2. Materials and methods

2.1. Animals and feeding protocols

A total of 28 male Wistar albino rats (n = 7, age: 8 weeks, weight:180 ± 20 g) housed in a controlled standard laboratory environment (12:12-h light-dark cycle at 22 °C) and animals fed with rat chow and water ad libitum. All animal procedures were approved by the Animal Experimentation Ethics Committee of Firat University (Elazig, Turkey). Table 1 provides a composition of the basal (control) diet. Rats were randomly divided into four groups: (i) sedentary control (no exercise and no CAPs), (ii) sedentary control + CAPs [no exercise + CAPs (0.2 mg capsaicinoids from 10 mg/kg BW/day Capsimax®), (iii) exercise, (iv) exercise + CAPs. CAPs administered as 0.2 mg capsaicinoids from 10 mg/kg BW/day Capsimax® daily for 8 weeks. Capsimax consists of capsaicinoids (CAPs) obtained from dried red fruits of Capsicum annuum L. The product (Capsimax) contains standardized 2% capsaicinoids (wt/wt), of which 1.2–1.35% is capsaicin, 0.6-0.8% is dihydrocapsaicin, and 0.1-0.2% is nordihydrocapsaicin. Capsaicinoids concentrate (Item code: 3822; Batch No: CFEB-07070003) was provided by OmniActive Health Technologies, Ltd., India. The dosage was chosen based on previously reported dosage in rodents [34].

Table 1
Composition of basal diet.

Description	%
Cracked Barley	30.2
Soybean meal	10.0
Sunflower meal	38.0
Wheat bran	6.0
Molasses	10.0
Limestone	3.0
Salt	1.5
DL-Methionine	0.8
Dicalcium phosphate	0.3
Vitamin and mineral premix[a]	0.2
Analysis (dry matter bases)	
ME, kcal/kg	3.088
Crude protein, %	24.3
Ether extract, %	3.4
Crude fiber, %	6.9
Ash, %	8.1
Ca, %	1.3
P, %	0.9

[a] The vitamin-mineral premix provides the following (per kg): all-trans-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-a-tocopherol acetate, 125 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B-6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B-12, 0.02 mg; folic acid, 0.55 mg; d-biotin, 0.1 mg. manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

2.2. Exercise protocol

The exercise protocols performed on a motor-driven rodent treadmill (MAY-TME, Commat Limited, Ankara, Turkey). The treadmill included a stimulus grid at the back end of the treadmill which provided an electric shock if the animal placed its paw on the grid. The apparatus consisted of a 5-lane animal exerciser utilizing single belt construction with dividing walls suspended over the tread surface. All exercise tests were performed during the same time period of the day to minimize diurnal effects. All rats were pre-trained in order for the animals to be exposed to the treadmill equipment and handling for 1 week. The exercise period based on (i) 1st day, 10 m/min, 10 min, (ii) 2nd day, 20 m/min, 10 min, (iii) 3rd day, 25 m/min, 10 min, (iv) 4th day, 25 m/min, 20 min and (v) 5th day, 25 m/min, 30 min. After 1-week treadmill familiarization to eliminate novel and stress effects, animals in treadmill exercise groups ran on the treadmill 25 m/min, 45 min/day and five days per week for 8 weeks according to the protocol published earlier [8]. None of the rats were subjected to harm during the endurance tests in this study.

2.3. Sample collection

At the end of the experiment, all rats were subjected to overnight fasting and blood and slow-twitch muscles (soleus and gastrocnemius