Reduction of Body Weight by Capsaicin is Associated with Inhibition of Glycerol-3-Phosphate Dehydrogenase Activity and Stimulation of Uncoupling Protein 2 mRNA Expression in Diet-induced Obese Rats

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Abstract

Capsaicin is a pungent component of red pepper, which is widely consumed as food adjuncts. The present study was performed to investigate anti-obesity effects of capsaicin in diet-induced obese rats. Male Sprague-Dawley rats (n=14) were fed with a high-fat diet (Control) or high-fat diet containing 0.016% capsaicin (w/w) (Capsaicin) for 8 weeks. The final body weight and the mass of white adipose tissue were significantly lower in capsaicin supplemented group compared to control. Dietary capsaicin ameliorated lipid profiles with decrease in the plasma concentrations of triglycerides and total cholesterol, and decrease in the levels of total lipids and triglycerides in the liver. Activity of glycerol-3-phosphate dehydrogenase (GPDH), an indicator of triglyceride biosynthesis in white adipose tissue, decreased by 35% in the group supplemented with capsaicin. However, consumption of capsaicin increased the expression of uncoupling protein 2 (UCP2) in white adipose tissue, which is related to energy consumption. Our data suggests that capsaicin may reduce body weight and fat accumulation in high fat diet-induced obese rats. These effects may be mediated, at least partially, by the upregulation of UCP2 gene expression and its ability to inhibit GPDH activity.

Key words: capsaicin, obesity, lipid metabolism, UCP2 expression, GPDH activity

INTRODUCTION

Obesity is caused from excess storage of triacylglycerol in adipose tissue due to the disequilibrium between energy intake and expenditure. Recently, prevalence of the obese population has rapidly increased at an alarming rate and is now a major worldwide health epidemic. Obesity is known as a risk factor for chronic diseases such as diabetes, hypertension, hyperlipemia, sclerosis of the arteries, and some forms of cancer (1,2). Recently, research has been conducted on capsaicin, a natural, anti-obesity substance that prevents fat storage and accelerates fat disintegration, which may have application to weight control and obesity (3-6).

Red pepper (*Capsicum annuum* L.) is used widely as a pungent spice due to alkaloid compounds, such as capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), which comprises 0.1~1% of red peppers (7-9). Previous studies have indicated that capsaicin treatment increased thermogenesis by accelerating the secretion of catecholamines from the adrenal medulla (10,11). Catecholamines interact with adrenergic receptors in the liver or adipose tissue and increase energy expenditure by stimulating lipolysis and thermogensis (12,13). Capsaicin increases en-

ergy expenditure and decreases the accumulation of body fat in laboratory rats fed a high fat diet (14,15) and in humans (16,17). Also, capsaicin inhibits cell differentiation and induces apoptosis in 3T3-L1 adipocytes (4). In diabetic mice, dietary capsaicin was reported to decrease obesity-induced insulin resistance via regulation of PPARα and TRPV-1 expression (18).

Uncoupling protein (UCP) is a mitochondrial proton transporter that uncouples oxidative phosphorylation; this activity has been proposed to influence thermogenesis and may act as an important controlling factor for body temperature maintenance, energy usage, and obesity. UCP2 was discovered as a structural homologue of UCP1, which is expressed mainly in brown adipose tissue (BAT), playing a crucial role in nonshivering heat production and is expressed in a wide variety of tissues in the body. UCP2 is not generally responsible for adaptive thermogenesis, but nonetheless, it might be significantly thermogenic when activated fully by endogenous or exogenous environmental effectors (19). Glycerol 3-phosphate dehydrogenase (GPDH) is an enzyme that catalyzes the reversible redox conversion of dihydroxyacetone phosphate to sn-glycerol-3-phosphate and plays a major role in lipid biosynthesis (20). Recent studies

in our laboratory elucidated that capsaicin increased the hydrolysis of triglyceride in 3T3-L1 adipocytes through regulation of multiple genes expressions that are involved in lipid catabolism and thermogenesis, such as hormone sensitive lipase (HSL), carnitine palmitoyl transferase-1α (CPT1-α), and uncoupling protein 2 (UCP2) (3).

Though the anti-obesity effects of capsaicin are well known through various mechanisms, the effects on the inhibition of obesity mediated by regulation of UCP2 gene expression and GPDH activity have not yet been fully elucidated. As a consequence, this study was designed to verify the effects of dietary capsaicin on the body weight gain and lipid metabolism in diet-induced obese rats. We measured the lipid profiles in serum and liver under capsaicin supplementation. In addition, the mRNA level of UCP2 related to thermogenesis, and the activity of GPDH, an indicator of adipogenesis, were measured in white adipose tissue.

MATERIALS AND METHODS

Animals and diets

14 Sprague-Dawley rats, 3 weeks of age, were obtained from Central Lab. Animal Inc. (Seoul, Korea) and housed individually in stainless steel wire mesh cages in a room kept at $22 \pm 1°C$ with a 12-hour light/dark cycle (light period: $08.00 \sim 20.00$ hr). They were fed laboratory chow and had access to water *ad libitum* for 1 week to stabilize their metabolic condition. After 1 week adaptation, the rats were randomly divided into 2 groups of 7 animals each and subsequently maintained on one of the following diets: a high-fat control diet (45% of total energy) containing 23% (w/w) fat, 17% (w/w) casein, 12% (w/w) sucrose, 20% (w/w) starch, 15% (w/w) dextrose, 4% (w/w) cellulose, 4.3% (w/w) minerals and 1.2% (w/w) vitamins, or high-fat control diet supplemented with 0.016% (w/w) capsaicin (Fluka Co., Buchs, Switzerland; Tokyo Chemical Industry Co., Tokyo, Japan) (Table 1). The diets were based on a modification of the AIN-93 diet and supplied gratis by Dyets Inc. (Bethlehem, PA, USA). The rats were maintained on these diets for 8 weeks. Body weight and food intake were monitored twice a week. All animal procedures conformed to NIH guidelines as stated in the Principles of Laboratory Animal Care (21).

Sampling procedures

At the end of the experiment, animals were anesthetized, a central longitudinal incision was made into the abdominal wall and blood samples were collected by cardiac puncture. Blood samples were centrifuged at

Table 1. The composition of experimental diets[1]

(in g/100 g diet)

Component	Control	Capsaicin
Casein	17.1	17.1
Cornstarch	20.2	20.184
Dyetrose	15.5	15.5
Sucrose	12.2	12.2
Cellulose	6.0	6.0
Lard	23.0	23.0
Mineral Mix[2]	4.3	4.3
Vitamin Mix[3]	1.2	1.2
L-Cystine	0.2	0.2
Choline bitartrate	0.3	0.3
Capsaicin	−	0.016
Total	100	100
Fat, % (calories)	45	45

[1]These diets were based on AIN-93G diet composition and given in powder form.
[2]AIN-93G Mineral Mix.
[3]AIN-93G Vitamin Mix.

$1,500 \times g$ for 20 min at 4°C and the plasma was separated and stored at -20°C until analyzed. Liver and white adipose tissue samples were excised, immediately frozen in liquid nitrogen and stored at -70°C.

Blood biochemical analysis

The plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, high-density lipoproteins (HDL) and triglyceride were determined by enzymatic colorimetric methods using commercial kits (Asan Pharmaceutical, Seoul, Korea).

Hepatic lipid analysis

Hepatic lipid was extracted using the method of Bligh and Dyer (22). The aliquots of hepatic lipid extracts were used for analysis of hepatic lipid profiles. The concentrations of triglyceride, total cholesterol and high density lipoprotein (HDL) cholesterol were determined by enzymatic colorimetric method using commercial kit (Asan pharmaceutical).

GPDH activity assay

Glycerol-3-phosphate dehydrogenase (GPDH)-specific activity was measured with a GPDH activity assay kit (Takara, Kyoto, Japan) in accordance with the manufacturer's instructions. Epididymal white adipose tissue was homogenized and centrifuged for 10 min at 4°C. GPDH activity was assayed in the supernatant by monitoring the decrease of NADH in the presence of dihydroxyacetone phosphate and measuring absorbance at 340 nm. Protein concentration was determined using a BCA protein assay kit (Thermo Scientific, Pittsburgh, PA, USA). Values for GPDH activity were expressed as a percentage of control.

Quantitative real-time RT-PCR

Total RNA was isolated from epididymal white adipose tissue using Trizol reagent (Invitrogen, Carlshad, CA, USA). The cDNAs were synthesized from 4 µg of RNA using M-MLV reverse transcriptase (Invitrogen). After cDNA synthesis, quantitative real time PCR was performed in 25 µL of Universal SYBR Green PCR Master Mix (Qiagen, Chatsworth, CA, USA) using a fluorometric thermal cycler (Rotor-Gene TM 2000, Corbett Research, Mortlake, Australia). Reaction mixtures were incubated for an initial denaturation at $95^{o}C$ for 15 min, followed by 30 cycles at $95^{o}C$ for 15 sec, $55^{o}C$ for 10 sec and $72^{o}C$ for 10 sec. Primers were designed using an on-line program (Primer3; http://frodo.wi.mit.edu/). The sequences of the designed primers were as follows: UCP2-sense-5'-ACTGTCGAAGCCTACAAGAC-3' and antisense-5'-CACCAGCTCAGTACAGTTGA-3', β-actin-sense-5'-GGCACCACACTTTCTACAA-3' and antisense-5'-AGGTCTCAAACATGATCTGG-3'. The ΔC_T method was used to measure relative quantification. The ΔC_T value for each sample was determined by calculating the difference between the C_T value of the target gene and the C_T value of the β-actin reference gene. The normalized target gene expression level in the sample was calculated by using the formula $2^{-\Delta\Delta CT}$. Values were expressed as fold change over control.

Statistical analysis

All data are expressed as mean±standard error of the mean (SEM) for the 7 animals in each group. The significant statistical difference between control and capsaicin-fed groups was analyzed by independent t-test using the SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). $p<0.05$ indicates a significant difference.

RESULTS

Body weight, energy efficiency ratio and fat accumulation

Body weight and energy efficiency ratio of the experimental groups are shown in Fig. 1. At the beginning of the experiment, the initial body weight was not significantly different between groups. After 8 weeks of capsaicin treatment, the final body weight of the group supplemented with capsaicin was significantly lower than that of the control group (Fig. 1A). The weights of different types of white adipose tissues are shown in Fig. 2. The group of rats that were fed with capsaicin showed significantly lower epididymal (24%) and retroperitoneal (25%) adipose mass than the control rat. There was no difference in the energy efficiency ratio between the groups over the study period, which showed that the
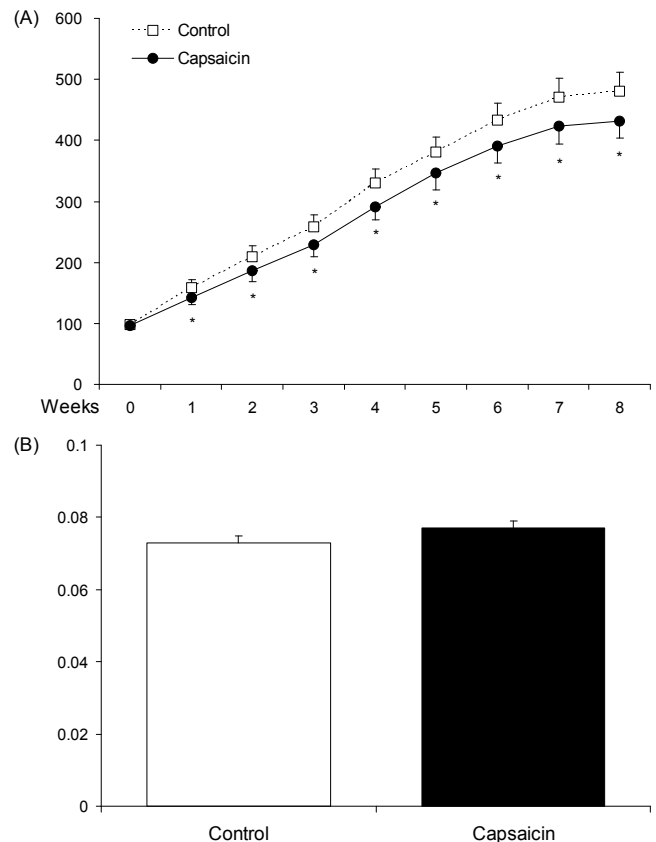

Fig. 1. Effect of capsaicin on body weight (A) and energy efficiency ratio (B). Energy efficiency ratio was calculated as the body weight gain per energy intake. Data are the mean ±SEM ($n=7$). Asterisks indicate significant differences compared with the control group, $^{*}p<0.05$.

beneficial effects of capsaicin on body weight and the weight of adipose tissues were not caused by reduced energy intake (Fig. 1B).

Blood and liver lipid profiles

The levels of triglyceride, total cholesterol and HDL cholesterol in plasma and liver are shown in Table 2. Rats on a capsaicin-containing diet showed significantly decreased plasma concentrations of triglyceride and total

Table 2. Effect of dietary capsaicin on plasma and hepatic lipid profiles

	Control	Capsaicin
Plasma lipids (mmol/L)		
Triglycerides	2.90±0.18	2.05±0.15[*]
Total cholesterol	1.98±0.20	1.49±0.08[*]
HDL cholesterol	0.99±0.10	0.97±0.05
Liver lipids (mg/g liver)		
Total lipid	39.07±3.78	28.40±0.97[*]
Triglycerides	13.43±1.08	10.07±0.75[*]
Total cholesterol	8.32±0.73	8.78±0.26
HDL cholesterol	0.19±0.04	0.12±0.02

Values are expressed as the mean±SEM ($n=7$). Asterisks indicate significant differences compared with the control group, $^{*}p<0.05$.
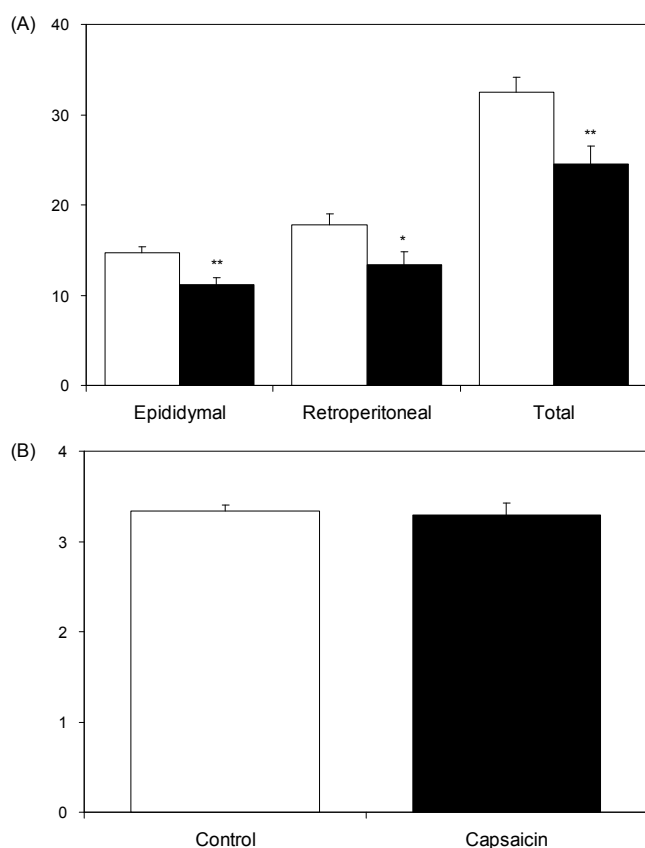
Fig. 2. Effect of capsaicin on adipose tissue weight (A) and liver weight (B). Data are the mean±SEM (*n*=7). Asterisks indicate significant differences compared with the control group, *p<0.05.

Fig. 3. Effect of dietary capsaicin on AST and ALT plasma activities. Values are expressed as the mean±SEM (*n*=7).
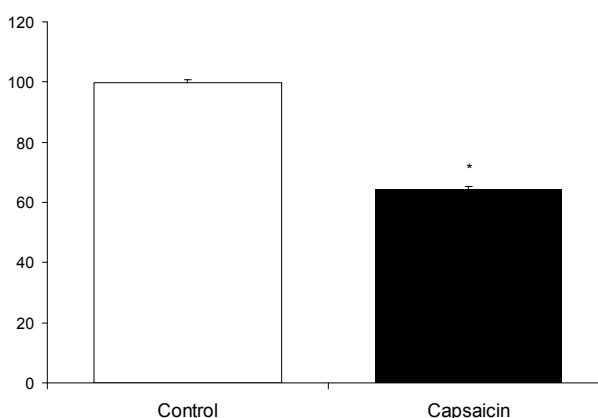
Fig. 4. Effects of capsaicin on GPDH activity in adipose tissue. GPDH activity was measured using a GPDH kinase kit and normalized to the protein content. Values are expressed as the mean±SEM (*n*=7). Asterisks indicate significant differences compared with the control group, *p<0.05.

cholesterol by 29% and 25%, respectively, compared with the control group. There was no significant difference in the level of plasma HDL cholesterol between the control group and the capsaicin supplemented group. In addition, the amounts of total lipids and triglyceride in the liver were significantly lower in the capsaicin-supplemented rats than the control rats. There was no significant difference in the levels of liver total cholesterol and HDL cholesterol between the control group and the capsaicin supplemented group.

Liver weight, plasma AST and ALT activities

At specific doses, capsaicin did not induce hepatic toxicity because the plasma activities of AST and ALT were within the normal range for rat, at 66.8～73.8 IU/L and 25.9～30.7, respectively (Fig. 3). In addition, liver weight and plasma levels of AST and ALT were unaffected by capsaicin treatment, which indicated that capsaicin was tolerated well by the animals (Fig. 2B).

GPDH activity

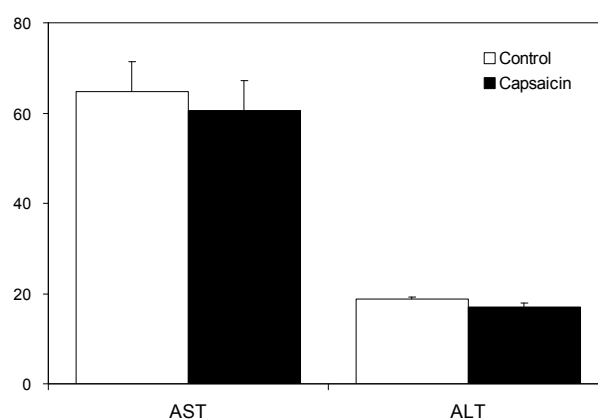In view of the role of GPDH inactivation in the biosynthesis of triglyceride, with the resultant anti-obesity effects, the effect of capsaicin on GPDH activity was determined in white adipose tissue (WAT). Activity of GPDH in white adipose tissue decreased by 35% in the group supplemented with capsaicin compared to control group (Fig. 4).

mRNA level of UCP2

To elucidate the mechanism by which capsaicin decreases the weight of WAT, we measured the mRNA level for the gene that is related to thermogenesis for WAT. The level of UCP2 mRNA in rats supplemented with capsaicin was 2.22-fold higher compared to the level expressed in control (Fig. 5).

DISCUSSION

In this study, we examined the effects of capsaicin on the development of obesity due to a high fat diet in rats. The dose of 0.016% capsaicin used in our study was determined based on previous studies related to anti-obesity effects of dietary capsaicin (15,18). The retroperitoneal adipose tissue weight and plasma triglyceride
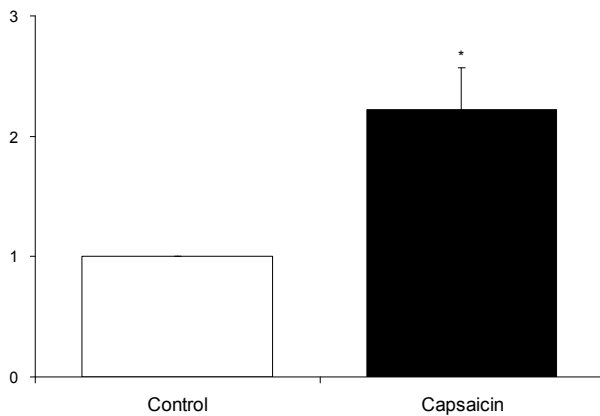
Fig. 5. Effects of capsaicin on the gene expression of UCP2. The mRNA of UCP2 was measured by quantitative real-time PCR. Values are expressed as the fold-change over control and as the means±SEM ($n = 7$). Asterisks indicate significant differences compared with the control group, *$p < 0.05$.

concentration decreased as the level of capsaicin increased up to 0.021% in rats fed a high fat diet (15). The dose of 0.016% capsaicin used in this study was not hepatotoxic for rats, showing that plasma levels of AST and ALT were unaffected by capsaicin supplementation. In a previous study, the serum activities of AST and ALT were not significantly affected by dieting with 0.02% capsaicin supplementation for 4 weeks in Sprague-Dawley rats (23). Our study showed that dietary capsaicin effectively reduced body weight and mass of various white adipose tissues. No significant difference was observed with respect to energy intake between rat fed the control diet and those fed a diet supplemented with capsaicin. These observations imply that dietary capsaicin did not cause an anorectic effect, which could have been responsible for the prevention or reduction of the increases in body weight and WAT that were induced by the high fat diet. Adiposity is associated with metabolic syndrome and cardiovascular diseases, and central obesity characterized as excessive visceral adipose tissue is more risky than peripheral obesity (24). The weight of visceral adipose tissue correlated strongly with total body weight and adiposity (25). Thus, the reduced body weight, which could be attributed partially to a decrease in the mass of the white fat pads in the capsaicin-supplemented group, reflected a marked anti-obesity effect of capsaicin. Zhang et al. (26) showed that mice on a high fat diet with 0.01% capsaicin had significantly lower body weight compared to that of mice on a high fat diet. A previous study suggested that the activation of transient receptor potential vanilloid type-1 (TRPV1) channels by capsaicin prevented adipogenesis and obesity (26). In another study, the body weight of obese mice treated with 0.015% capsaicin was significantly lower than that of obese mice on a high fat

diet (18). Several *in vitro* studies reported that the treatment of capsaicin on 3T3-L1 adipocytes inhibited adipogenesis and increased lipolysis or apoptosis (3,5). Capsaicin decreased the intracellular lipid content and up-regulated mRNA levels of genes involved in lipid catabolism such as hormone sensitive lipase (HSL), carnitine palmitoyl transferase-Iα (CPTI-α) and uncoupling protein 2 (UCP2) in 3T3-L1 adipocytes (3).

Various reports suggesting beneficial effects of capsaicin on plasma lipid parameters have been published to date, although some reports question its therapeutic use (26,27). We have demonstrated herein that capsaicin reduced the plasma concentration of triglycerides and total cholesterol. These findings are in agreement with previous studies, which have shown an inverse correlation between dietary capsaicin and the concentrations of plasma cholesterol and triglycerides. Zhang et al. (26) showed that plasma triglycerides levels of mice on a high fat diet plus capsaicin were significantly decreased compared with that of mice on a high fat diet. Kawade et al. (15) reported that a 0.014% capsaicin supplemented diet reduced the level of serum triglyceride but not serum total cholesterol. Moreover, our data showed that the amounts of total lipid and triglycerides in the liver were also lower in capsaicin-treated rats compared to control. Manjunatha and Srinivasan (27) reported that 0.015% capsaicin supplementation decreased the levels of serum total cholesterol and triglyceride, and hepatic triglyceride. A previous study suggested that the cholesterol- lowering effects of capsaicin stem in part from activation of hepatic cholesterol 7α-hydroxylase, the rate-limiting enzyme converting cholesterol to bile acids, which is an important pathway for eliminating cholesterol from the body (28).

Body weight is regulated by the balance between energy intake and consumption. Our data showed a decrease in body weight by capsaicin treatment without any significant difference in energy intake, which suggests that dietary capsaicin has a physiological effect on the process of energy expenditure. To understand the mechanisms that underlie the anti-obesity action of capsaicin, we analyzed the mRNA level of UCP2 involved in thermogenesis in WAT. UCP2 is expressed in various tissues, such as the liver, brown adipose tissue (BAT), WAT, skeletal muscle, kidney, and lung, and also in the immune system (29). Although UCP2 was proposed to function in adaptive thermogenesis in a manner equivalent to UCP1, much evidence indicates that UCP2 primarily attenuates the mitochondrial production of reactive oxygen species, which protects against oxidative damage (19). However, fatty acids, superoxides and alkenals activate UCP2 when thermogenesis is required,

which suggests that the activation of UCP2 by physiological activators might cause significant thermogenesis under certain conditions (19). For these reasons, UCP2 remains an attractive thermogenic target for the treatment of obesity (19). In our study, capsaicin treatment increased the expression level of UCP2 significantly in WAT. GPDH activation can regulate energy metabolism that favours the stimulation of lipogenesis by conversion of dihydroxyacetone phosphate to sn-glycerol-3-phosphate (20). In the present study, capsaicin supplementation decreased activity of GPDH in WAT. Also, the inactivation of GPDH may have led to the decreased amounts of WAT in rats treated with capsaicin. In 3T3-L1 adipocytes, capsaicin was reported to decrease the amount of intracellular triglycerides and GPDH activity (5).

In conclusion, dietary supplementation with capsaicin resulted in reduced body weight and mass of WAT and ameliorated plasma lipid profiles in rats with obesity induced by a high fat diet. These effects were mediated, at least partially, by downregulating the expression of the UCP2 gene that is involved in thermogenesis. This study suggests that the anti-obesity effects of capsaicin result from its ability to inhibit GPDH activity.

ACKNOWLEDGEMENTS

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