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Capsinoids Supplementation does not Prevent Weight Gain and does not Change Lipid Profile in Wistar Rats Fed a High-Fat Diet

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Abstract We hypothesized that capsinoids supplementation, a bioactive vanillylamines isolated from chili peppers, would exert protective effects against high-fat diet -induced weight gain via regulation of lipid, glucose and insulin profile. To test our hypothesis, twenty-four male Wistar rats were fed a standard diet, standard diet with capsinoids, high-fat diet and high-fat diet with capsinoids for 6 weeks. Capsinoids dose was 0.18 mg/kg/d. During the experiment, body weight and food intake were evaluated weekly and biochemical analyses were performed at the end of the experimental period. Only high-fat diet with capsinoids presented lower total food intake as compared to standard diet with capsinoids group (~35%). Nevertheless, there were no significant differences in weight gain between the groups. Capsinoids supplementation did not prevent retroperitoneal and epididymal fat gain on high-fat diet with capsinoids, as well did not change brown fat and liver weight. Moreover, no statistical difference was observed for high-fat diet or capsinoids supplementation on blood glucose, insulin and lipid profile. In conclusion, these results suggest that capsinoids supplementation in obesity experimental model of Wistar rats, has limited effects to prevent weight and fat gain, as well as do not regulate metabolic profile.

Keywords: *capsinoids, metabolic profile, obesity, Wistar rats, high-fat diet*

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1. Introduction

Obesity has reached epidemic proportions and is currently a public health problem in Brazil as well as in the world. The evidence-based literature describes obesity as a chronic medical condition of multifactorial etiology, including genetic, environmental, metabolic and behavioral factors [1].

Data from the World Health Organization showed that in 2016 more than 1.9 billion adults, 18 years of age or older, were overweight and over 650 million were obese [2]. Obesity is associated with the development of comorbid conditions such as type 2 diabetes, hypertension, hepatic steatosis, coronary heart disease, among others [3].

Intervention programs focusing on inducing a negative energy balance with diet or exercise, or both, are effective in inducing weight loss and weight loss maintenance in the short to medium term but lose efficacy in the long term. [4,5]. Recently, there has been a growing demand for spice-based drugs, because they have fewer adverse effects and have antioxidant and anti-inflammatory properties, thus indicating benefits in obesity. Among them, Capsicum annum, present in red pepper, has been investigated for its lipid-lowering, anti-diabetic and anti-obesity effects [6,7].

Red pepper, with the scientific name of Capsicum annuum, belongs to the Solanaceae family of the genus Capsicum, and is not pungent due to the absence of alkaloid capsaicin in its composition. Red pepper refers to different plants with common names including chili pepper, tabasco pepper, African chilies, cayenne pepper, paprika and also christmas pepper [8]. The Capsicum Dry Extract is extracted from Capsicum annuum, a sweet pepper species. It is composed of 3 capsinoids (capsiate, dihydrocapsiate and nordiidrocapsiate), non-pungent, found in all variants of the genus Capsicum plant. They

are structurally identical to the pungent constituents of Capsicum, i.e., capsaicin, dihydrocapsaicin and nordihydrocapsaicin, respectively [9, 10]. Several studies indicated that red pepper and its active constituent, capsaicin, have therapeutic potential in different components of metabolic syndrome.

Study on rats fed high-fat diet for 8 weeks showed that capsaicin significantly decreased triglyceride level [11]. Another study conducted by Otunola et al. [12], in male Wistar rats indicated that the administration of 200 mg/kg of the aqueous extract of red pepper improved weight gain after 4 weeks. Moreover, it was observed lower levels of total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), atherogenic index and elevated serum high density lipoproteins (HDL) [12].

Several studies have demonstrated that capsaicin plays an important role in a number of pathophysiological processes through the activation of the transient receptor potential vanilloid subfamily member 1 (TRPV1) and increased secretion of catecholamines, increasing thermogenesis and reducing weight gain and adipogenesis [13, 14]. Capsaicin may further reduce obesity-related glucose intolerance and reduce the gene expression of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) [15]. In addition, capsaicin increases the gene expression of adiponectin, reducing the inflammatory response of adipose tissue [15].

Data from a study by Haramizu et al [16] showed that accumulation of body fat in human was suppressed after 2 weeks of capsinoids treatment. However, Okumura et al [17] showed that capsaicin supplementation did not reduce weight gain and accumulation of white adipose tissue in KK-A (y) diabetic rats, despite the reduction of blood glucose.

Thus, in view of the above and considering the epidemic proportions of obesity and the urgency of new strategies for its prevention, the present study aimed to investigate the effects of capsinoids on weight gain, lipid and glucose profile and insulin of rats fed a high-fat diet.

2. Materials and methods

2.1. Animals and Treatment

This study was experimental, prospective and longitudinal. Twenty-four male Wistar mice (6–8 weeks after birth) were obtained from the Central Animal Facilities of the Ribeirão Preto Campus, University of São Paulo. The animals were kept in cages under controlled conditions of 12 h Dark-Light cycles and constant temperature (25 ± 1°C) with water and diet ad libitum for two weeks to stabilize their metabolic condition. After the 2-week adaptation, they were randomly separated into four groups of six animals each: standard diet (SD), standard diet supplemented with capsinoids (SDC), high-fat diet (HFD) and high-fat diet supplemented with capsinoids (HFDC).

The supplemental dose was 0.18 mg/kg/day by insertion of capsinoids into powder mixed in food. Capsici Dry Extract was extracted from Capsicum annuum (purity of 40% (w/w) of capsinoids) and was purchased from local market. The animals from HFD diet groups were fed the diet composed of 400 g of standard chow, 100 g of sucrose, 100 g of lard, 170 ml of soybean oil, 400 g of milk powder and 0.04 g of butylated hydroxytoluene. The animals from standard diet group were fed a commercial Nuvilab CR1 chow, based on the recommendation of the American Institute of Nutrition, AIN-93, for growing rodents. The nutritional composition of the standard diet and the high fat diet are described in Table 1.

Table 1. Nutritional composition of the standard and high-fat diets (g/100g diet)

	Standard[a]	High-fat[b]
Carbohydrates	44.8g (42%)	48.47g (34.5%)[c]
Lipids	3.4g (4%)	31.85g (51%)[d]
Proteins	24.8g (22%)	20.37g (14.5%)
Energy (Kcal/weight)	309 Kcal	562 Kcal

[a]Standard. Source: Nuvilab. (NUVILAB-CR1 Nuvital-Colombo, Brazil), contains the following nutritional composition: protein 22%; lipids 4%; carbohydrate 42%; minerals 10%; phosphorous 0.8%; vitamins 1%; fibers 8%; humidity 12.5%. To the bromatological analysis, 100g of dry matter of the diet contained: 309kcal; 24.8 protein; 3.4g lipids; 44.8g carbohydrate; 8.2g fixed mineral residue; 18.8g dietary fiber.
[b]High-fat. Source: Brazilian Food Composition Table - TACO Version 4, contains the following composition: 34.55% standard chow, 8.13% sucrose, 13.32% lard, 32.55% powdered milk and 12.44% soybean oil.
[c]The kind of carbohydrates of the High-Fat diet: sucrose and lactose.
[d]The kind of lipids of High-Fat diet: polyunsaturated fatty acids and saturated fatty acids.

The total period of treatment with capsinoids (associated with a HFD or SD) and follow-up of the diets of the animals that did not use the supplementation were six weeks.

The body weight was checked before and every week during the intervention period using a digital scale with a maximum capacity of 15 kg (Filizola S.A., São Paulo, Brazil. The percentage of weight gain was calculated by the difference between the final weight and initial weight using the following equation 1:

$$\frac{[(finalweight - initialweight)]x100}{initialweight} \quad (1)$$

The dietary intake of rats was recorded daily, by means of the difference between the amount of food offered and the amount remaining in the feeder on the following day, which allowed the determination of the 24-hour food intake.

Glucose concentration was measured with a glucometer (One Touch - Johnson & Johnson) in the first week of treatment (initial) and on the day of euthanasia (final).

At the end of the experiment, the animals were euthanized by decapitation and blood samples were collected for insulin and lipid profile analysis. During the euthanasia, the blood was collected and promptly centrifuged at 3500 rpm, 4 ºC for 15 minutes to obtain the serum, which was kept in a freezer at -70 ºC for subsequent biochemical analysis. Samples of hepatic tissue, epididymal adipose tissue, retroperitoneal adipose tissue and brown adipose tissue were collected, weighed and immediately maintained in liquid nitrogen to be stored in a freezer at -70 °C.

The experimental protocol was approved by the Animal Research Ethics Committee of Ribeirão Preto Medical School, University of São Paulo (protocol no. 020/2013) and the ethical principles that have their origins in the Declaration of Helsink.

2.2. Biochemical Analysis

Insulin analysis was performed by the commercial Elisa Ultrasensitive Kit (Mouse Ultrasensitive Insulin ELISA, Alpco Diagnostics, Salem USA). This insulin kit is a Sandwich-Type immunoassay, where each microplate is coated with insulin-specific monoclonal antibody of all animals. Total cholesterol, HDL and triglycerides were analysed by enzymatic method using commercial kits from Labtest (Labtest Diagnóstica S.A., Brazil).

2.3. Statistical Analysis

Data are presented as means ± standard error (SE). Normality test (Kolmogorov-Smirnov test) was followed by nonparametric Kruskal-Wallis and post-hoc Dunn test to identify differences between groups. Data analysis was performed using IBM SPSS v.22 software. A significance level of 5% was adopted.

3. Results

3.1. Body Weight and Food Intake in Experimental Groups

HDFC group had lower total food intake, approximately 35%, as compared to SDC group (Figure 1B). No statistical changes were found in weight gain, however, HFD and HDFC showed approximately 15 and 10% less weight gain, as compared their respectively standard diet groups (SD and SDC) (Figure 1D).

3.2. Biochemical Analysis in Experimental Groups

Blood measurements of glucose, insulin and lipid profile are shown in Table 2. No statistical difference was observed for high fat diet or capsinoids supplementation groups.

Table 2. Plasma biochemical profile (analysis at the end of study)

Variables	SD	SDC	HFD	HFDC
Glucose (mg/dL)	124 ± 7.9	107 ± 30	139 ± 5	132 ± 20
Insulin (ng/dL)	0.6 ± 0.2	0.12 ± 0.1	0.91 ± 0.3	0.41 ± 0.1
TG (mg/dL)	196 ± 93	185 ± 83	244 ± 75	179 ± 42
TC (mg/dL)	134 ± 95	130 ± 31	185 ± 89	157 ± 47
LDL (mg/dL)	65 ± 111	52 ± 30	97 ± 81	85 ± 19
HDL (mg/dL)	40.3 ± 15	49 ± 8	60 ± 23	59 ± 21

TG: triglycerides; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein. Data are mean ± SD. All the groups were analysed by Kruskal-Wallis followed by post-hoc Dunn test ($p < 0.05$), n= 6 each group. SD: standard diet, SDC: standard diet plus capsnoids, HFD: high-fat diet, HFDC: high-fat diet plus capsinoids.

3.3. Tissue Weight in Experimental Groups

The HFDC had higher retroperitoneal fat pad compared with SDC group, approximately 96%. Similar magnitude effect was observed for HFD when compared with SD group, but did not achieve statistical difference (Figure 2A). Although HFD have presented significant increase in epididymal fat pad (~57%) as compared to SD group, this effect did not present statistical difference (Figure 2B).

In respect of brown fat pad, HFD had higher values as compared to SD (~50%), however, this comparison did not present statistical difference. Capsinoids supplementation induced a slight decrease of brown fat pad on the HFDC as compared to HFD (~28%), however, this comparison did not achieve statistical difference (Figure 2C). Capsinoids supplementation did not prevent liver weight gain on HFDC when compared to SDC group. In this comparison, HFDC had an increase on the liver weight around 46% (Figure 2D).

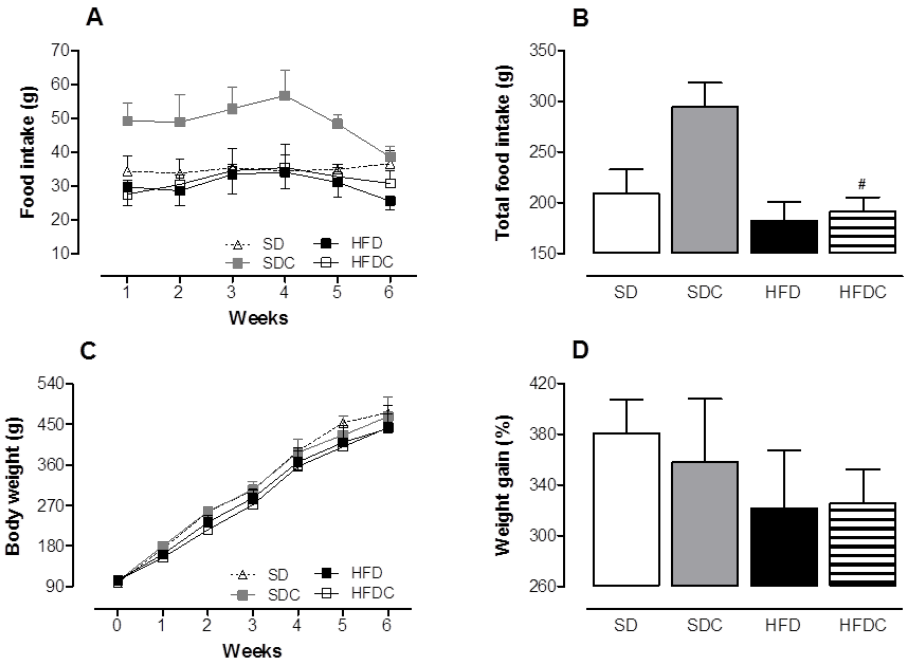

Figure 1. Food intake and body weight of rats fed with standard and high-fat diet with and without capsinoids. #different from SDC

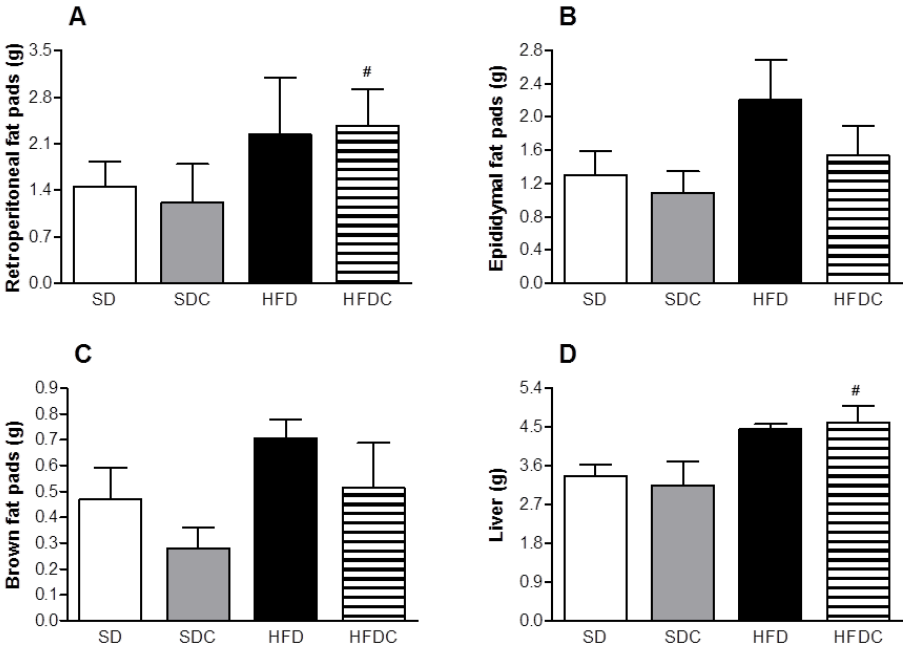

Figure 2. Tissue weight of rats fed with standard and high-fat diet with and without capsinoids. #different from SDC

4. Discussion

The main findings of the present study were that capsinoids supplementation did not prevent weight and fat gain. In addition, capsinoids supplementation did not change serum glucose, TG, TC, and LDL, even though HFDC group presented lower total food intake.

Our results confirm the findings of a previous study in which supplementation of oleoresin capsicum (OC) for 14 weeks in HFD group, found no significant difference in body weight [18]. Moreover, the authors also observed that TG and TC level were also not affected by OC supplementation. It was suggested that OC has lower water solubility, thermal stability, oral bioavailability and these effects reduce physiological performance of this active compound [18].

It was observed in previous studies that HFD compared to SD group, exhibited markedly higher body weight and capsinoids could prevent huge body weight gain [19]. These results were controversial in our study, because HFD and HFDC groups presented less weight gain when compared to SD and SDC respectively. We did not expect these results on weight gain. However, our analysis found a remarkably variability in body weight and did not observe statistical difference between groups.

During the feeding period, food intake was higher in the SD than in the HFD groups. Similar food behavior was demonstrated previously [19]. This result can be attributed to high caloric density of HFD in comparison to SD.

Regarding organs weight, we observed a slight decrease in epididymal fat pad on HDFC (approximately 30%), but without statistical significance. Sung, Jeong and Lee [19] provided HFD associated with Capsicum annuum L. supplementation (10 or 100 mg/kg doses) for 7 weeks in mice. It was noted that epididymal fat pad was significantly lower in HFD with capsinoids as compared to mice without supplementation.

We did not observe capsinoids effects in retroperitoneal fat pad. Yeon et al., [20] who performed capsinoids supplementation in ICR mice fed with HFD, also did not find statistical difference in retroperitoneal fat pad. Despite not observing fat pad changes, Yeon et al., [20] showed that all adipose tissue of HFDC group was consistently lower than HFD. In our study the fat pad response to capsinoids was similar, however, the variability of the data did not allow to observe any effective changes. It is suggested that capsinoids binds with TRPV1 which induces secretion of catecholamines, promotes lipolysis, suppresses fat accumulation and increase energy metabolism [21]. Changes in adipose tissue caused by capsinoids are effective when this compound suppresses adipogenesis [7]. Capsinoids acts regulating early phase events of adipogenesis by ~~via~~ activating the 5'-adenosine monophosphate-activated protein kinase pathway [7].

In relation to liver weight, we did not observed significant difference between the HFD and HFDC. Other studies managing capsinoids supplementation in HFD mice models, have demonstrated the same patterns in liver weight [19, 20].

Regarding ~~to~~ lipid metabolism, we did not observe HFD or capsinoids supplementation effects. Previous study has shown that capsinoids did not prevent increase on plasma TG and TC. However, capsinoids supplementation can increase HDL which eliminates excess cholesterol from tissues [20]. Controversially, Kim and Park [22] provided HFD to C57BL/6 mice combined with green pepper supplementation (Capsicum annuum L.) and demonstrate that green pepper exerted a significant reduction in TG and TC concentration. It is discussed that an ingredient of Capsicum annum L., such as capsaicin, might inhibit serum triglyceride accumulation due to anti-inflammatory properties in experimental models [22]. Is worth to note, that lipid metabolism is influenced by body weight decrease. We hypothesized that to observe any difference in lipid profile in our study, a decrease in body weight would be necessary.

The strength of the present study is that, to the best of our knowledge, it is one of the few studies in Brazil that investigated the effect of capsinoids on body weight and metabolic profile in Wistar rats. Some limitations in our study include relatively small sample size and the short intervention period. According to the results, we suggest that further studies are necessary, increasing the period of supplementation and evaluating it is on histological analysis and gene expression of pro and anti-inflammatory cytokines.

5. Conclusions

In conclusion, our results suggest that capsinoids supplementation in obesity experimental model of Wistar rats has limited effects to prevent weight and fat gain. Moreover, capsinoids did not affect glucose or lipid metabolism.

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