Ingested capsaicinoids can prevent low-fat–high-carbohydrate diet and high-fat diet-induced obesity by regulating the NADPH oxidase and Nrf2 pathways

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Objective: Capsaicinoids (CAPs), most commonly found in chili peppers, have a multitude of pharmacological and physiological effects, such as anti-inflammation, antioxidant, and anticancer effects. In the present study, we set out to investigate the hypothesis that CAPs mitigate obesity in rats and the possible mechanisms thereof.

Materials and methods: Rats were divided into six groups, including control (±10 mg CAPs/kg body weight [BW]), low-fat–high-sucrose diet (±10 mg CAPs/kg BW), and high-fat diet (±10 mg CAPs/kg BW). Blood samples and liver and aortic tissues were taken at the end of the study.

Results: CAPs supplementation significantly reduced hyperglycemia and hyperlipidemia (P<0.001) and ameliorated oxidative damage by reducing malondialdehyde concentrations in serum and liver and by increasing total antioxidant capacity in serum induced by the low-fat–high-sucrose and high-fat diets (P<0.001 for all). CAPs also depressed levels of NFκB p65, gp91phox, p22phox, and Nrf2, Sirt1, and endothelial nitric oxide synthase were significantly increased in the aorta.

Conclusion: CAPs may at least partially reduce adverse effects due to high-fat diet and sucrose consumption through regulation of energy metabolism, oxidative stress, and proteins involved in vasoprotection.

Keywords: capsaicinoids, metabolism, oxidative stress, lipid profile, antioxidant capacity

Introduction

Metabolic syndrome is a worldwide health problem with increased morbidity and comprises a group of risk factors such as insulin resistance, obesity, hyperglycemia, hypertension, and dyslipidemia.¹-³ Metabolic syndrome has been reported to develop due to such factors as a surplus of fat and simple carbohydrates from high-fat diets (HFDs) and high-sucrose diets (HSDs).²,³ Experimental models have been studied in detail by our research group on the metabolic changes that result from the consumption of these diets. As a result of our and other studies, these diets have been reported to trigger insulin resistance and promote inflammatory processes, hyperinsulinemia, and hyperlipidemia, and increase blood pressure, hepatic steatosis, and vascular dysfunction.⁴-⁶ In addition, an association between HFDs and HSDs facilitating oxidative stress and a reduction in nitric oxide levels has been revealed.⁶ In recent years, many studies have been conducted to determine the potential effects of phytochemicals, such as cinnamon, genistein, resveratrol, and capsaicin, on animal models induced...
by HFDs. However, there have been no reports on the effects of the molecular mechanism of capsaicinoids (CAPs) in HFDs and HSDs in rats.

CAPs are the major pungent, naturally occurring active compounds in capsicum fruit, such as hot chili peppers (genus Capsicum), with the most abundant forms being capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. The available information indicates that CAPs possess a wide variety of biological and physiological properties, including anti-inflammatory, antioxidant, and anticancer. Whiting et al. indicated that CAPs played a beneficial role as part of a weight-management program. Capsicum extract is a CAP-enriched standardized product obtained from dried red fruit of C. annuum. CAPs are responsible for the spicy taste of the chili pepper berry. They are hydrophobic, colorless, odorless, and crystalline waxy compounds, and their varieties are present in capsicum. The CAPs in the pepper are biosynthesized from branched-chain amino acids and phenylalanine in pepper fruit. In chili pepper, capsaicin is the primary CAP, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Capsicum spp. have been used as a carminative, digestive irritant, stomachic, stimulant, rubefacient, and tonic. The plants have also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps, and toothache. It has also been reported that consumption of capsaicin promotes fat oxidation in negative energy balance and did not increase blood pressure significantly. Additionally, capsiate activates TRPV1 receptors in the gut. Capsicum extract is also used in cosmetic products, where it functions as a skin-conditioning agent.

### Materials and methods

#### Animals

A total of 42 male Wistar rats (age 8 weeks, weight 180±20 g) were housed in a controlled standard laboratory environment (12:12-hour light:dark cycle at 22°C) and fed with rat chow and HFD diets, and had water ad libitum. All experiments were conducted under the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and approved by the ethics committee of Firat University, Elazig, Turkey. The composition of diets (control and HFD) is shown in Table 1.

#### Experimental diets and design

After 1 week of acclimation to a standard rodent-chow diet, 42 rats were randomly allocated into six groups, with seven rats in each group: controls, rats fed chow diet (12% of calories as fat); CAPs, rats fed chow diet and administered CAPs (Capsimax® [OmniActive Health Technologies, Ltd., Morristown, NJ, USA]; 10 mg/kg body weight [BW], 0.2 mg CAPs); HSD, rats fed chow diet plus 20% sucrose (30% w:v) in the drinking water; HSD + CAPs, rats fed chow diet plus 20% sucrose (30% w:v) in the drinking water and administered CAPs (Capsimax; 10 mg/kg BW, 0.2 mg CAPs); HFD, rats fed an HFD (42% of calories as fat); and HFD + CAPs, rats fed an HFD and administered CAPs (Capsimax; 10 mg/kg BW, 0.2 mg CAPs). Rats were treated orally with CAPs (10 mg/kg BW dissolved in 5% dimethyl sulfoxide) daily by oral gavage to the end of the experiment. Capsimax consists of CAPs obtained from dried red fruit of C. annuum L. The product is standardized to 2% CAPs, of which 1.2%–1.35% is capsaicin, 0.6%–0.8% dihydrocapsaicin, and 0.1%–0.2% nordihydrocapsaicin. CAP concentrate was provided by OmniActive Health Technologies, Ltd., (Morristown, NJ, USA). The dosage of CAPs chosen was based on previously reported dosage in rodents. Animals were administered CAPs for 8 weeks. HFDs were prepared weekly in our laboratory in pellets and stored at −4°C.

#### Sample collection

At the end of the experimental period, rats were weighed and then killed by decapitation. Blood samples were collected and serum was prepared by centrifuging the blood at 3,000 × g for 10 minutes, aliquoted into 1.5 mL vials, frozen at −80°C, and used for biochemical parameters and malondialdehyde (MDA) analyses. Livers and aortas were carefully

### Table 1 Composition of diets (g/kg) fed to rats

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Starch</td>
<td>579.5</td>
<td>150.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50.0</td>
<td>149.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70.0</td>
<td>0</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>0</td>
<td>400.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Vitamin–mineral premix</td>
<td>45.0</td>
<td>45.0</td>
</tr>
<tr>
<td>I-Cysteine</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Notes: The vitamin–mineral premix provides the following (per kg): all-trans-retinyl acetate 1.8 mg; cholecalciferol 0.025 mg; all-rac-α-tocopherol acetate 12.5 mg; menadione sodium bisulfate 1.1 mg; riboflavin 4.4 mg; thiamine mononitrate 1.1 mg; vitamin B6 2.2 mg; niacin 35 mg; Ca-pantothenate 10 mg; vitamin B12 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.
removed, weighed, and then stored at −80°C. Samples (1 g) were homogenized in 2 mL TBS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) and centrifuged at 3,000 × g for 15 minutes at 4°C. Supernatants were collected and used for MDA estimations.

**Laboratory analyses**

Serum parameters were determined by an automated analyzer (Labgeo PT10; Samsung Electronics Co, Seoul, South Korea). Levels of insulin and leptin were analyzed with rat insulin and leptin kits (Linco Research, Inc., St Charles, MO, USA) via an enzyme-linked immunosorbent assay device (Elx-800; BioTek Instruments, Inc., Winooski, VT, USA). The assay’s sensitivity was 0.18 and 0.26 ng/mL for insulin and leptin, respectively. Inter- and intra-assay constants were 5.2% and 6.1% for insulin and 4.7% and 6.5% for leptin, respectively. MDA concentrations in livers and aortas were measured as per the method in Karatepe via high-performance liquid chromatography with a Shimadzu (3-ethylbenzothiazoline-6-sulfonate) by antioxidants to its bis measured using dark blue–green color reduction 2,2'-azino-10 minutes at 4°C. Total antioxidant capacity (TAC) was measured using glucose (87% and 70%), insulin (138% and 61%), and HFD-fed rats treated with CAPs. No significant difference was found either in BW between HFD-fed rats and HFD-fed rats treated with CAPs (P > 0.05, Table 2). No significant differences were detected in safety end markers for liver-function tests in any treatment (P > 0.05, Table 2).

As seen in Table 3, HSD and HFD feeding increased serum levels of glucose (87% and 70%), insulin (138% and 61%), free fatty acid (FFA; 233% and 288%) and leptin (345% and 103%) in HSD and HFD rats, respectively (P < 0.001). Hypertriglyceridemia and elevated lipid indicators in HSD- and HFD-fed rats were reduced with CAP supplementation (49% and 43% compared to control rats, respectively).

**Statistical analysis**

Sample size was based on a power of 85% to obtain P < 0.05. Differences among groups were evaluated by analysis of variance and Tukey’s post hoc analysis. P < 0.05 was considered statistically significant. Data were analyzed in SPSS for Windows version 20 (IBM, Armonk, NY, USA).

**Results**

**Body weight and biochemical parameters**

The effects of CAP supplementation on final BW, lipid profile, and safety end markers for liver-function tests are shown in Table 2. HSD and HFD feeding increased final BW, total cholesterol and triglycerides by 10% and 6%, 56 and 49%, and 182% and 139% compared to control rats, respectively (P < 0.001). CAP treatment decreased total cholesterol and triglycerides by 17% and 26% and 12% and 19% in HSD- and HFD-fed rats, respectively (P < 0.001). No significant difference was found either in BW between HFD-fed rats and HFD-fed rats treated with CAPs (P > 0.05, Table 2). No significant differences were detected in safety end markers for liver-function tests in any treatment (P > 0.05, Table 2).

**Table 2** Effects of capsaicinoids (CAPs) on final BW and plasma biochemical parameters in high-sucrose diet (HSD)- and high-fat diet (HFD)-fed rats

<table>
<thead>
<tr>
<th>Item</th>
<th>Groups</th>
<th>Control</th>
<th>CAPs</th>
<th>HSD</th>
<th>HSD + CAPs</th>
<th>HFD</th>
<th>HFD + CAPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final BW, g</td>
<td></td>
<td>289.00±3.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>288.36±4.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>318.14±6.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>314.86±3.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>307.00±4.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>306.00±4.65&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>TC, mg/dL</td>
<td></td>
<td>62.14±1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.71±0.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97.43±1.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.29±1.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>92.86±2.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.71±1.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td></td>
<td>28.57±0.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.86±1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.71±2.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.43±1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.43±2.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.00±2.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td></td>
<td>75.43±3.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.71±3.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.43±3.85&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>76.86±4.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>AST, U/L</td>
<td></td>
<td>141.71±5.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139.00±4.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.71±6.51&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>146.7±3.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Notes:** Different superscript letters indicate group mean differences (P < 0.05). Data are means ± SE. **Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; BW, body weight; TC, total cholesterol; SE, standard error; TG, triglycerides.
by 18% and 16% for glucose, 8% and 14% for insulin, 43% and 52% for FFA, and 44% and 40% for leptin, respectively (P < 0.05). Serum and liver MDA levels increased 114% and 97% for HSD and 62% and 73% for HFD (P < 0.001, Table 3), and serum TAC decreased by 72% for HSD and 57% for HFD upon obesity induction. CAP treatment caused a 32% and 37% reduction for HSD and 16% and 18% reduction for HFD in serum and liver MDA concentrations and elevation in serum TAC by 163% and 54% in the HSD- and HFD-fed rats (P < 0.001), respectively, similar to the control group (P > 0.05).

### Aortic protein levels

Aortic NFKB, a transcription factor, gp91phox, and p22phox (essential components of NADPH oxidase) levels were increased by HFD and HSD intake, whereas HFD- and HSD-fed rats had decreased heart Nrf2, an emerging regulator of antioxidant enzymes, including SOD, CAT, and glutathione peroxidase (P < 0.0001 for all). However, CAPs decreased levels of NFkB, gp91phox, and p22phox and increased levels of Sirt1, Nrf2, eNOS significantly in aortas of CAP-treatment groups (P < 0.05, Figure 1, A–F).

### Discussion

In this study, we report that hyperglycemia, hypertriglyceridemia, p22phox, and gp91phox levels, the major components of NADPH oxidase in rats fed HFD and HSD, were increased in the aorta and that this rise was linked to increased lipid peroxidation and NF-kB activities and reduction of Nrf2, Sirt1, and eNOS activities. Supplementation of CAPs alleviate the adverse effects of HFD and HSD.

Capsicum has been shown to help improve metabolism and hormone function, stabilize blood glucose, and reduce insulin and leptin resistance. Capsicum and CAPs have also been linked to cardiovascular health, by improving endothelial function, and inhibiting low-density-lipoprotein cholesterol oxidation. Capsicum may also help prevent cancer, likely due to its antioxidant activity. The burning sensation associated with capsaicin results from its chemical interaction with sensory neurons. Capsaicin binds selectively to TRPV1, which resides on the membranes of pain- and heat-sensing neurons. Capsaicin causes an ion channel to open below body temperature, which is why capsaicin is linked to the sensation of heat. Prolonged activation of these neurons by capsaicin depletes presynaptic substance P, one of the body’s neurotransmitters for pain and heat. The changes observed in this study may have been due to its selective binding to TRPV1; however, further studies need to be carried out in humans. In the current study, it was observed that CAPs reduced total cholesterol, triglycerides, glucose, and insulin in both the HSD and HFD groups. In addition, we observed HSD and HFD enhanced all metabolic health risk factors and increased oxidative stress markers.

CAPs are potent free radical scavengers and prevent membrane stability. Kogure et al showed that capsaicin hindered MDA levels and ROS production in rat peritoneal macrophages, as well as inflammatory reactions in sepsis. In addition, chronic exposure to capsaicin depletes neurons of neurotransmitters, reduces pain sensation, and blocks inflammation. In the current study, CAPs increased potential antioxidant properties and demonstrated their ability to protect tissue from damage or inflammation. Similarly, in a previous study, we reported that CAP supplementation elevated the activity of SOD, CAT, and glutathione peroxidase and significantly decreased MDA levels in serum and ovaries. Kempaiah and Srinivasan reported that reduced liver antioxidant enzymes, including SOD, CAT, and glutathione peroxidase, in hypercholesterolemic rats were efficiently resisted by capsaicin supplementation (0.015%). Lee et al reported that capsaicin administration for three days reduced
Figure 1 Effects of capsaicinoids (CAPs) in high-sucrose diet (HSD)- and high-fat diet (HFD)-fed rats.

Notes: Aortic NFκB (A); Nrf2 (B); Sirt1 (C); endothelial nitric oxide synthase (eNOS) (D); p22phox (E); Gp91phox (F). The intensity of the bands was quantified by densitometric analysis. Data expressed as ratio of normal control value (set to 100%). Data are expressed as percentage of control value. Each bar represents the mean and standard error of mean. The intensity of the bands (G) was quantified by densitometric analysis. Blots were repeated at least four times, and representative blots are shown. β-Actin was included to ensure equal protein loading. Different superscripts denote significant differences between groups not sharing the same superscript (P<0.05).

Capsimax®; OmniActive Health Technologies, Ltd., Morristown, NJ, USA.
MDA concentration in liver, kidney, muscle, and lung in rats. It is also known that CAPs stimulate energy consumption by activation of the sympathetic nervous system, which induces catecholamine secretion from the adrenal medulla.10,41 This thermogenic influence has the advantage of weight management. CAPs have also been reported to decrease appetite,42–44 increase thermogenesis,28,45 and increase lipolysis, and FFA.10 Associations among consumption of CAP-containing food and low incidence of obesity have been reported earlier.10,46 CAPs reduce metabolic health risk factors causing obesity and increased energy metabolism. Sirt1 decreases in the HSD and HFD groups indicate that these diets increase the risk of obesity and cardiometabolic syndrome.

The current study revealed different regulatory pathways to prevent oxidative stress, improve antioxidant potential, and enhance energy metabolism (Figure 1). NFκB influenced inflammatory proteins, and CAPs decreased NFκB levels and enhanced Nrf2 activity. In addition, Sirt1 modulates various cellular processes that directly regulate glucose metabolism and stress metabolism, increases fat mobilization and energy metabolism, thereby controlling the direct chromatin structure and stimulating brown remodeling of the white fat in white adipose tissue, regulates pancreatic insulin secretion, detects nutrient availability in the hypothalamus, impairs obesity-induced inflammation in macrophages, and modulates circadian time activity in tissues.47 In the current study, CAPs increasing SIRT1 suggest its metabolic role in activating energy metabolism. Further studies are needed to explore this pathway. The thermogenic effect of CAPs is mediated at least in part by a CAP-sensitive structure located in the rostral ventrolateral medulla.48 Increased eNOS protein levels in CAP treatment suggest vasoprotection and assistance in reducing cardiovascular risk. CAP treatment may also stimulate vasodilatation,30 which may indirectly impact thermogenesis, as any resultant loss of heat may necessitate an increase in metabolism. A previous study showed that capsaicin upregulated HO1 expression by Nrf2 stimulation in HEPG2 cells.49

Many studies have shown that obesity, HFD intake, hyperglycemia, high tissue lipids, excessive angiotensin II production, and hyperleptinemia can cause vascular damage through numerous metabolic pathways, such as free-radical generation and inflammation.50,51 The inflammatory condition of obesity increases leukocyte tissue infiltration and free-radical production, while cytokines are known to upregulate the activity of redox enzymes, including NADPH oxidase (NOX).52 NOX is an enzyme system that contains membrane (gp91phox, p22phox) and cytosolic (p47phox, p67phox, p40phox) components and produces superoxide, which combines on the plasma membrane to form active oxidase.53 Previous studies have suggested that obesity-linked vascular dysfunction is facilitated by NOX-induced oxidative stress.51,54 Upregulation of NOX has been related to elevated ROS induced by angiotensin II and TNFα.55,56 In previous studies, it has been reported that high glucose incubation upregulates expression of the NADPH subunits p22phox and p67phox in bovine aortic endothelial cells51 and in microvascular endothelial cells.57 In the current study, the upregulated NADPH oxidase subunits p22phox and p67phox stimulated by HSD or HFD were decreased by CAP supplementation. The results of this study showed that CAPs might enhance the increased NADPH oxidase levels that result in increased ROS generation, whereas the increase in ROS generation is mostly responsible for the HFD- or HSD-induced endothelial hyperpermeability. Therefore, CAPs inhibited endothelial overpermeability by inhibiting NOX. In addition, Zuo et al51 reported that green-tea polyphenols protected against overexpression of high glucose-stimulated p22phox and p67phox. Akar et al58 reported that decreased relaxation to acetylcholine and intensified contractions to phenylephrine and angiotensin II were linked with downregulated eNOS and Sirt1, whereas gp91phox and p22phox proteins were upregulated and superoxide production triggered in aortas from high-fructose corn syrup-treated rats. They also reported that resveratrol supplementation efficiently restored high-fructose corn syrup-induced deteriorations.

Several proteins are altered by CAPs, many of which suggest antioxidant properties, reducing oxidative stress and increased metabolism. The results suggest that thermogenesis-and lipid metabolism-associated proteins were significantly changed upon capsaicin administration, suggesting that capsaicin may be a beneficial phytochemical for attenuation of obesity.53 CAPs also provide antioxidant activity, which may help protect against inflammatory diseases. In hamsters, CAPs support heart health by lowering cholesterol and increasing blood flow, presumably due to antioxidant activity.54 In another study, chili peppers not only reduced total and non-high-density cholesterol and triglycerides but were also related to reduced levels of compounds connected to inflammation, such as Cox2.59

This study further suggests that HSD and HFD diets increase cardiometabolic health risk factors, inflammation, and oxidative stress and reduce potential antioxidant capacity. Therefore, adding CAPs to these diets may help to regulate metabolism and improve antioxidant properties, including reducing metabolic and cardiometabolic health risk factors.
Further long-term clinical studies are required to show and explore similar findings in humans.

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**Disclosure**

VJ is an employee of OmniActive Health Technologies, Inc., NJ, USA. The authors report no other conflicts of interest in this work.

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