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Red peppers with different pungencies and bioactive compounds differentially modulate energy and glucose metabolism in ovariectomized rats fed high fat diets

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ABSTRACT

We hypothesized that differences in red peppers pungencies and bioactive compounds are associated with different effects on obesity and glucose tolerance, and tested the hypothesis in ovariectomized (OVX) rats fed high fat diets. Increasing red pepper pungency was associated higher concentrations of capsaicin, dihydrocapsaicin and total capsaicinoids; and lower concentrations of β-carotene, total carotenoids and chlorogenic acid. After 8 weeks of consuming 1% different types of red peppers, moderately and severely pungent red peppers (MSP and SSP) improved energy homeostasis better than less pungent red pepper (LSP): MSP and SSP increased energy expenditure and decreased visceral fat mass. This was related to elevated uncoupling proteins (UCP)-1, UCP-2 and UCP-3 expressions and decreased expressions of genes involved in fatty acid synthesis. LSP enhanced insulin sensitivity and improved hepatic insulin signaling. In conclusion, red peppers with different color and pungency differently modulate energy and glucose homeostasis in OVX rats fed high fat diets.

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Abbreviations: BMI, body mass index; OVX, ovariectomy; CPT-1, carnitine palmitoyltransferase-1; ACC, acetyl CoA carboxylase; SREBP-1c, sterol regulatory element-binding protein-1c; FAS, fatty acid synthase; LSP, less pungent red pepper; MSP, moderately pungent red pepper; SSP, severely pungent red pepper; TRPV1, transient receptor potential vanilloid type 1; RQ, respiratory quotient; OGTT, oral glucose tolerance test; PKB or Akt, protein kinase B; CREB, cAMP responding element binding protein; AMPK, AMPK kinase; PEPCK, phosphoenolpyruvate carboxykinase

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1. Introduction

Koreans have a much lower average body mass index (BMI) than most of the developed world with only 31.3% of the adult population having a BMI above 25 (Rhee, Park, Kim, & Woo, 2013). In contrast, among adult Americans 72.3% of men and 64.1% of women have BMIs of 25 or more (Flegal, Carroll, Kit, & Ogden, 2012). Undoubtedly, many dietary and lifestyle factors as well as genetic differences contribute to the difference, but the high consumption of red pepper in the Korean diet could be one contributing factor.

Koreans have traditionally consumed relatively large amounts of red pepper, which has been show to improve energy balance via elevating resting energy expenditure (Kim & Park, 2005; Yu et al., 2012). Many human and animal studies have investigated the modulation of energy intake and expenditure by capsaicin, a major component of red pepper (Kwon et al., 2013; Saito & Yoneshiro, 2013). Red pepper intake has increased from 5.2 g/day in 1998 to 7.2 g/day in 2005 in Korea, and the increased intake might be associated with the low prevalence of obesity in the Korean population. Kim and Park (2005) revealed that red pepper intake was positively correlated with energy intake ($r = 0.05$) in 100 female undergraduate students; but despite the increased energy intake, it was negatively correlated with waist circumferences ($r = -0.2$) and fat mass ($r = -0.2$) as measured by bioelectrical impedance analysis, suggesting that red pepper intake might decrease fat mass by increasing energy expenditure. In our previous study (Kwon et al., 2013) 0.025% capsaicin intake decreased body weight gain, visceral fat accumulation, and serum leptin levels without modulating energy intake in diabetic rats. In addition, capsiate, a non-pungent capsinoid, also improved energy homeostasis. Snitker et al. (2009) reported that oral intake of 6 mg/day of non-pungent capsinoids resulted in abdominal fat loss by increasing fat oxidation in 40 obese men and 40 obese women. Another non-pungent capsinoid, dihydrocapsiate (3 or 9 mg/day), is reported to have caused a small thermogenic effect of about 50 kcal/day for 1 month in seventy-eight healthy men. Although no study has reported a direct effect on energy homeostasis, serum levels of β-carotene, another red pepper component, are negatively associated with abdominal fat mass in children (Canas et al., 2012) and β-carotene suppresses fat accumulation in 3T3-L1 adipocytes (Lobo et al., 2010). Overall, all of the major red pepper components including capsaicinoids, capsinoids, and carotenoids have the potential to modulate energy and glucose metabolism in experimental animals and humans.

Different varieties of red peppers contain different combinations of carotenoids, capsaicinoids and capsinoids which give the peppers various colors and pungencies and possibly different effects on energy and glucose homeostasis. However, few studies have investigated the effects of different types of red pepper on energy and glucose homeostasis in experimental animals. Therefore, we hypothesized that different varieties of red peppers having more or less pungency and different colors due to carotenoid content might differently modulate energy and glucose homeostasis in diet-induced obese rats. The objective of the study was to test the hypothesis using three varieties of red pepper from Young Yang County (Gungsangbuk-Do, Korea) according to the intensity of pungency: less pungent red pepper (LSP; Geumdang), moderately pungent red pepper (MSP; Chilsung) and severely pungent red pepper (SSP; Subicho) in ovariectomized (OVX) rats fed a high fat diet and also to explore possible mechanisms. OVX rats have similar symptoms as post-menopausal women with increased visceral fat mass and bone loss (Santollo, Wiley, & Eckel, 2007), making them a good model for studying dietary interventions for preventing obesity and related pathologies.

2. Materials and methods

2.1. Extraction and lyophilization

Three varieties of red pepper powders (LSP, MSP, and SSP) were extracted with 70% ethanol by shaking for 24 h at 25 °C, centrifuged, and supernatants lyophilized (Il Shin) 8000*g* for 30 min.

2.2. Analysis of bioactive compounds

Bioactive components in the red pepper extracts were analyzed by HPLC using a YMC ODS-AM (250 mm × 4.6 mm I.D.; particle size, 5 μm) reversed-phase column (JASCO-ChromNAV, Japan). The mobile phase consisted of solvents, 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). The following gradient was used: initial 0 min A:B (88:12, v/v); 18 min A:B (78:22); 28 min A:B (72:28); 35 min A:B (62:38); 48 min A:B (52:48); 54 min A:B (32:68); 58 min A:B (0:100); 60 min A:B (0:100); and 62 min A:B (88:12). The mobile phase flow rate was 1.0 mL/min, the column temperature was 35 °C, the injection volume was 20 μL, and UV detection was at 285 nm. The major compounds in the 70% ethanol extracts of red peppers were quantified using different standards including 20 flavonoids (Sigma Co.), 8 carotenoids (Sigma Co.), capsaicin, dihydrocapsaicin, capsiate, and other compounds, and their contents were calculated from standard curves based on each of the standards (2–10 μg/mL) using ChromNAV. Total phenolic compounds were analyzed using Folin–Ciocalteu reagent and expressed as mg gallic acid equivalents • g^{-1} (Singleton, Orthofer, & Lamuela-Raventós, 1999). Total carotenoids were analyzed with a modified method of Ritter and Purcell (1981) and expressed with the following equation using the specific extinction coefficient ($E^{1\%}_{1mL}$) of 2072 for capsanthin. Total flavonoids were measured by the modified methods of Davis (1947) with rutin as the standard.

2.3. Experimental animals and design

Female Sprague–Dawley rats, weighing 239 ± 12 g, had either ovariectomy or sham operations and were housed individually in stainless steel cages in a controlled environment (23 °C and a 12-h light and dark cycle). All surgical and experimental procedures were performed according to the NIH Guidelines and were approved by Hoseo University Animal Care and Use Review Committee. Experimental animals freely consumed water and their respective diets during the eight-week experimental period. The high fat diet was a modified semi-purified AIN-93 formulation (Reeves, Nielsen, & Fahey, 1993) consisting of 40 energy percent (En%) carbohydrates, 20 En% protein, and 40 En% fats. The major carbohydrate,