Nutrition 32 (2016) 129–137

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Methyl jasmolate treated buckwheat sprout powder enhances glucose metabolism by potentiating hepatic insulin signaling in estrogen-deficient rats

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ARTICLE INFO

Article history:
Received 3 May 2015
Accepted 21 July 2015

Keywords:
Methyl jasmolate
Buckwheat sprout
Energy expenditure
Glucose
Bone mineral density

ABSTRACT

Objectives: Methyl jasmolate (MeJA)-treated vegetables produce higher concentrations of various bioactive compounds. We investigated whether long-term oral consumption of MeJA-treated and untreated buckwheat sprout powder improves energy, glucose, lipid, and bone metabolism induced by estrogen deficiency in ovariectomized (OVX) rats fed high-fat diets, and explored the mechanisms involved.

Methods: OVX rats were divided into four groups and fed high-fat diets supplemented with 3% dextrin (OVX-control), buckwheat sprout powder (BWS), or MeJA-treated buckwheat sprout powder (MJ-BWS) for 12 wk. Sham rats without estrogen deficiency had a control diet as a normal-control.

Results: MeJA-treatment increased total polyphenols and flavonoids by about 1.6 fold and isoorientin, orientin, rutin, and vitexin were elevated by about 18% in buckwheat. After 12 wk, OVX rats exhibited increased weight gain, fat mass, skin temperature, hyperglycemia, and decreased bone mineral density (BMD) compared to sham rats. BWS prevented the increase of skin temperature and decrease of femur BMD, but did not improve energy glucose homeostasis as much as MJ-BWS. MJ-BWS prevented increases in body weight and fat mass. Energy expenditure was lowest in OVX-control, followed by BWS, MJ-BWS, and normal-control. Furthermore, MJ-BWS exhibited greater improvements in glucose and insulin tolerance than OVX-control and BWS. Phosphorylation of hepatic Akt and AMPK was potentiated, in ascending order of OVX-control, BWS, MJ-BWS, and normal-control, whereas PEPCK expression was decreased.

Conclusions: MJ-BWS prevented and ameliorated the disturbances in energy and glucose metabolism in estrogen-deficient animals better than BWS. Therefore, besides flavonoids in BWS, other components such as phytoalexins produced in MJ-BWS during MeJA-treatment might play a crucial role in the improvement.

Introduction

Over the past 25 y, the importance of social, emotional, and physical changes in women's health during midlife have been increasingly emphasized and interventions sought to improve long-term health and well-being. These changes are related to the decrease in estrogen experienced by menopausal women, and to various diseases that affect life expectancy and quality [1]. Estrogen deficiency impairs energy, glucose, lipid, and bone metabolism, and can lead to the development of metabolic diseases such as obesity, type 2 diabetes, cardiovascular diseases, and osteoporosis [2,3]. Estrogen treatment is used to prevent the onset of metabolic diseases in postmenopausal women [4], but it increases the risk of breast and endometrial cancers, venous

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† These authors contributed equally to this manuscript. This research was supported by Korea Food Research Institute and High Value-added Food Technology Development Program, Ministry of Agriculture, Food and Rural Affairs and the Korea Science and Engineering Foundation in Korea in 2014.

http://dx.doi.org/10.1016/j.nut.2015.07.012
0899-9007/

thromboembolism, and stroke [5]. Due to its adverse effects, the potential to substitute estrogen with phytoestrogens, which are natural products originating from plants that are structurally or functionally similar to estradiol, has been evaluated [6].

Buckwheat (*Fagopyrum esculentum*) and buckwheat sprout (BWS) are consumed as foods. In Asia, sprouts (mainly from beans) have been used in various dishes. They contain higher amounts of nutrients, such as vitamin C, protein, and phytochemicals when compared to seeds. Recently, BWS has become a more popular ingredient in salads in Korea. BWS is rich in flavonoids and its major bioactive compound is rutin (quercetin-3-*O*-rutinoside) [7]. Rutin has beneficial effects on high-fat induced dyslipidemia, hyperglycemia, and cognitive impairment, possibly due to its amelioration of inflammasome activation [8–10]. Inflammation is associated with the pathogenesis of obesity and insulin resistance [11].

When a pathogen attacks, buckwheat's defense system is activated. It produces hormones such as jasmonic acid, ethylene, abscisic acid, and salicylic acid to resist and overcome the attack [12]. Growing evidence indicates that methyl jasmolate (MeJA) significantly increases the production of numerous phytoalexins, which represent highly valuable secondary metabolites of pharmaceutical and industrial importance [13,14], and that play a role in plant defense against fungal and other microbial pathogens. Known phytoalexins, such as resveratrol, exhibit potent bioactivity against metabolic diseases [15]. Under stress from MeJA, BWS may induce phytoalexin production and modulate existing BWS phytochemicals. Studies conducted by our research team demonstrate that exogenous MeJA treatment does not modulate sprout growth, but MeJA-treated buckwheat sprouts (MJ-BWS) do exhibit significantly higher amounts of phenolic compounds such as isoorientin, orientin, rutin, and vitexin, and greater antioxidant activity compared to untreated BWS [16]. Therefore, natural products treated with plant hormones such as jasmonate, and its methyl ester, can produce bioactive compounds such as phytoalexins that may be associated with superior prevention and alleviation of metabolic diseases.

As both MJ-BWS and BWS contain polyphenols (particularly flavonoids), they may act as phytoestrogens that prevent and/or reduce metabolic dysregulation in postmenopausal women. However, the efficacy of MJ-BWS might be better than that of BWS. Therefore, we hypothesized that long-term oral consumption of MJ-BWS and BWS powders would ameliorate impairments in energy, glucose lipid and bone metabolism induced by estrogen deficiency. We also explored the mechanisms of action underlying this effect in ovariectomized (OVX) rats fed a high-fat diet.

Materials and methods

Buckwheat sprout cultivation and MeJA treatment

Buckwheat (*Fagopyrum esculentum Moench*) seed harvested in 2012 was obtained from the Pyeongchang County Office (Kangwon, Korea), where a voucher specimen was deposited. Buckwheat seeds were washed and soaked in distilled water at 25°C for 4 h. The seeds were then placed in a tray and covered with a cheese cloth. Four separate trays were placed in a commercial sprout cultivator (MikroFarm, Easy Green, Cody, WY, USA) using an autospray system. The sprouts were cultivated in the dark at 18 ± 2°C for 7 d, with water automatically sprayed for 30 min every 12 h. For the application of MeJA to the sprouts, 0.1 mM MeJA dissolved in 0.25% ethanol was sprayed on them once per day. As an untreated control, 0.25% ethanol was sprayed on a different set of sprouts. The sprouts were harvested at 0 and 7 d of cultivation. Samples on day 0 represented sprouts before MeJA treatment. The harvested sprouts were immediately lyophilized and ground into powder, which was then stored at −70°C for use in the animal study.

Total phenol, flavonoid and rutin content of BWS and MJ-BWS sprout powders

The total phenolic compounds in the buckwheat sprouts were determined using Folin-Ciocalteu's reagent according to a published method [17]. The two types of buckwheat sprouts were extracted in methanol (1:20 w/v), and 0.5 mL of the sample extract was mixed with 1 N Folin-Ciocalteu's reagent with an equal volume and held for 3 min at room temperature (25°C). The mixture was then added to 10 mL of 2% sodium carbonate solution and reacted for 1 h. The absorbance of solution was measured at 750 nm, using a UV-spectrophotometer (V-570, Jasco Co., Japan). A standard curve was prepared using gallic acid (GA, Sigma Chemical Co., Louis, MO, USA), and the absorbance was converted to phenolic content expressed as milligrams of GA equivalent (GAE) per gram of dry weight.

The extracts were dissolved in ethanol and total flavonoid contents were measured using the modified methods previously described [18]. About 0.1 mL of sample extract was mixed with 0.15 mL of 5% sodium nitrite solution (1:1.5 v/v) and held for 6 min at room temperature (25°C). The mixture was then, added to 0.3 mL of 10% aluminum chloride solution and reacted for 5 min at room temperature. The absorbance of the solution was measured at 415 nm, using a UV-spectrophotometer (V-570, Jasco Co., Japan). A standard curve was prepared using rutin hydrate (Sigma Chemical Co., Louis, MO, USA).

The isoorientin, orientin, rutin, and vitexin contents of MJ-BWS and BWS sprout powders were analyzed using high-performance liquid chromatography (HPLC). Each extract was filtered with 0.45-μm membrane filter and analyzed using a Nano 2-D LC system (Shimadzu, Kyoto, Japan). A Discovery C18 column (250 × 4.6 mm, 5 μm; Supelco, Bellefonte, PA, USA) was used, and the mobile phase consisted of (A) acetic acid:water = 2:98 and (B) acetic acid:acetonitrile:water = 2:45:53. The initial flow rate was set (A) at 50% and decreased to 0% after 18 min and (A) was then increased to 50% after 2 min, and was maintained at 50% for the final 2 min. The total analysis time was 22 min. The oven temperature was set at 35°C and a flow rate was 1 mL/min. An injection volume of 10 μL was used, and absorbance was determined using a UV detector set at a wavelength of 355 nm. The amount of each flavonoid was quantified using respective standards such as isoorientin, orientin, rutin hydrate, and vitexin (Sigma Chemical Co., Louis, MO, USA). The chromatogram of the BWS and MJ-BWS and the chemical structure of isoorientin, orientin, rutin hydrate, and vitexin are given in Figure 1. The calibration curves were found to be linear over the 0.92 to 91.7 μg/mL, 0.77 to 76.9 μg/mL, 0.85 to 86.4 μg/mL, and 0.92 to 92.4 μg/mL range studied for isoorientin, orientin, rutin, and vitexin, respectively, with correlation coefficients (r) for each analytics >0.998. The limit of detection for compounds presented ranged from 0.17 to 1.08 mg/mL and the limit of quantification was in the range of 0.53 to 3.29 mg/mL. All major peaks were identified and quantified.

Animals

Female Sprague–Dawley rats weighing 227 ± 18 g were housed individually in stainless steel cages in a controlled environment (23°C and with a 12/12 h light/dark cycle). All surgical and experimental procedures were performed according to the guidelines of the Animal Care and Use Review Committee of Hoseo University, Korea. Experimental diets were made with a high-fat diet containing 3% BWS powder or 3% MJ-BWS powder. The high-fat diet was prepared using a modified AIN-93 semipurified formulation specific to experimental animals [19]. The diets in all groups consisted of 40% energy (En%) from carbohydrates, 20 En% from protein and 40 En% from fats. Based on the results of the nutrient composition analysis of the BWS and MJ-BWS powders (Table 1), starch, casein, soybean oil, and cellulose were removed from the high-fat diet so that the composition of each diet was identical aside from their polyphenol content. The major carbohydrate, protein, and fat sources were starch plus sugar, casein (milk protein), and lard (CJ Co., Seoul, Korea), respectively. The composition of the diet of each group is shown in Table 2.

Experimental design

Rats underwent ovariectomy or a sham operation under anesthesia induced by intramuscular injection of a mixture of ketamine and xylazine (100 and 10 mg/kg body weight, respectively). A midventral incision was made, and the ovary was isolated by ligation of the most-proximal portion of the oviduct [20]. The ovary was then removed with scissors. This procedure was repeated on the contralateral side. The same procedure was carried out for the sham groups, except for the removal of the ovaries [20]. Thirty OVX rats were randomly assigned to the following three groups: 1) BWS, 2) MJ-BWS, and 3) OVX-control. As the normal-control group, 10 sham-operated (sham) rats were assigned to a high-fat diet without supplementation. The rats were provided with the assigned the diet for 12 wk. Overnight-fasted serum glucose levels, food, water intake, and body weight were measured every Tuesday at 1000 h. Insulin resistance was determined as the homeostasis model assessment of insulin resistance (HOMA-IR) by calculating from fasting serum glucose and insulin concentrations [21].