Abstract

The postmenopausal state is associated with an increased risk of metabolic syndrome. Grape seed extract (GSE), a rich source of proanthocyanidins, has beneficial effects on low-grade inflammatory diseases in a high-fat diet-induced obesity model and therefore is a candidate to improve postmenopausal metabolic abnormalities. In the present study, the effects of dietary GSE on body weight and glucose tolerance were examined in ovariectomized (OVX) mice. Female C57BL6/J mice underwent ovariectomy or a sham operation, and OVX mice were orally administered GSE (100 mg/kg B.W.) or the vehicle solution (OVX+V). Mice in the OVX+V group weighed significantly more than mice in the Sham group. Body weight was significantly lower in the OVX+GSE group than in the OVX+V group. The weights of subcutaneous white adipose tissue (WAT) and visceral WAT were significantly lower in the OVX+GSE group than in the OVX+V group. In glucose tolerance tests, OVX+GSE mice showed significantly lower blood glucose levels at 30 min and 60 min than those in OVX+V mice. In conclusion, we demonstrated that GSE prevents metabolic disorders, such as obesity and glucose intolerance, in OVX mice. These results support the potential therapeutic applications of GSE in postmenopausal women.

Key Words: proanthocyanidins, postmenopause, obesity, glucose tolerance

Introduction

The postmenopausal state is associated with an increased risk of metabolic disorders, such as hyperuricemia, hypertension, and type 2 diabetes. These metabolic disorders might be caused by both the direct effects of estrogen deficiency and secondary effects of obesity. Postmenopausal women gain weight due to a temporary increase in food consumption and reductions in energy expenditure. Premenopausal women tend to store fat in a subcutaneous depot. However, fat deposition shifts to favor the visceral depot, leading to an increased prevalence of metabolic syndrome in postmenopausal women.

Grape seed is one of the richest sources of proanthocyanidins, which are polymers of flavan-3-ols with an average degree of polymerization between 2 and 17. Previously, we showed that grape seed extract (GSE) has anti-oxidative activity, including the ability to scavenge free radicals. Procyanidin B3 eluted from GSE prevents osteoarthritis-related changes via anti-oxidative effects. It has also been suggested that GSE has anti-inflammatory, anti-diabetic, anti-carcinogenic, and anti-aging effects. Additionally, GSE has beneficial effects in low-grade inflammatory diseases in a high fat diet-induced obesity model.
that GSE intake could improve estrogen deficiency-induced metabolic abnormalities. In the present study, we examined the effect of dietary GSE on body weight and glucose tolerance in ovariectomized (OVX) mice.

Methods and Procedures

Animal experiments
All animal experiments were approved by the Animal Care and Use Committee of Tokyo Medical and Dental University. Seven-week-old female C57BL/6J mice were obtained from Sankyo Labo Service (Tokyo, Japan) and used after acclimatization to their new environment for 1 week. Animals were housed at an approximate temperature and relative humidity of 23–25°C and 70%, respectively, under a 12-h light-dark cycle. All the animals were allowed unrestricted activity and were provided food and water ad libitum. Animals underwent ovariectomy or a sham procedure, and were divided into the following three groups: (i) sham-operated (Sham) mice administered the vehicle orally, (ii) OVX mice administered the vehicle orally (OVX+V), and (iii) OVX mice administered GSE (100 mg/kg) orally (OVX+GSE). GSE or vehicle were given orally every day from the day after the surgical operation. Ovariectomy was carried out by making two small incisions on the back of each mouse to remove both ovaries under anesthesia with medetomidine hydrochloride, midazolam, and butorphanol tartrate. The sham operation was carried out in the same manner, except that the ovaries were not excised.

Test substances
GSE (Leucoselect®) was obtained from Indena (Milan, Italy); Leucoselect has a well-defined chemical composition elucidated completely by instrumental analyses (e.g., high-performance liquid chromatography–mass spectrometry) and contains >80% proanthocyanidins. There is no potential conflict of interest to disclose.

Glucose and insulin tolerance tests
Mice were fasted overnight (16 h) before glucose tolerance tests were performed at 6th week after the operation (14-week-old). In brief, glucose (1 mg per g body weight; Sigma–Aldrich, St. Louis, MO, USA) was orally administered to mice to assess glucose clearance. Blood samples were collected from the tail vein before and 30, 60, 90, and 120 min following the injection. Blood glucose levels were measured using Glutest Neo-Alpha (Sanwa Kagaku Kenkyusho Co., LTD., Nagoya, Japan).

Weighing of visceral adipose tissue depots
Thirteen weeks after surgery, all mice were sacrificed and inguinal white adipose tissues (inguinal WAT), uterine WAT, mesenteric WAT, and brown adipose tissues (BAT) were excised and immediately weighed. To evaluate skeletal muscle weight, quadriceps muscles were excised and weighed.

Food intake measurements
Two weeks after surgery, food intake for 24 h was estimated by subtracting the amount of food left in the cage dispenser and the amount of food dropped on the cage floor from the initial food weight.

Statistical Analysis
To compare the three groups, as the data does not follow normal distribution, we performed multiple comparison analysis with non-parametric Steel’s many-one rank test (two sided test and one sided test), setting OVX+V group as a control; p ≤ 0.05 was used as the threshold for statistical significance. Results are expressed as means ± SD. Statistical analysis was performed using BellCurve for Excel ver.2.00 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results
OVX+V mice weighed significantly more than mice in the Sham group. In comparison to the OVX+V group, the OVX+GSE group had a significantly reduced body weight (Figure 1A).

Weighing of visceral adipose tissue depots
Thirteen weeks after surgery, all mice were sacrificed and inguinal WAT, uterine WAT, mesenteric WAT, and brown adipose tissue (BAT) were excised and immediately weighed. The weights of subcutaneous WAT and visceral WAT were significantly higher in the OVX+V group than in the sham group. In comparison to the OVX+V group, the OVX+GSE group exhibited lower subcutaneous WAT and visceral WAT weights. The weights of the BAT, quadriceps muscle, liver, and pancreas were not significantly different in all three groups (Figure 1B).

Food intake measurements
We monitored food intake in female mice 2 weeks after the surgical operation. After 24 h, food intake was higher in the OVX+V group than in the sham group. Food intake in the OVX+V group was not significantly different to that of the OVX+GSE group (Figure 1C).

To explore the effect of GSE administration on glucose homeostasis and insulin sensitivity, glucose tolerance tests were performed for all groups. OVX+V mice showed higher glucose levels than those in sham mice at 30 min and 60 min following glucose administration.
Effect of grape seed extract on metabolic syndrome

Figure 1.

(A) Left: Body weights of the sham, OVX+vehicle, and OVX+GSE groups at the indicated number of weeks after the operation. Right: Body weights of the sham, OVX+vehicle, and OVX+GSE groups at 13th week after the operation when sacrificed.

(B) Weight of the indicated organs of the sham, OVX+vehicle, and OVX+GSE groups. *p < 0.05 vs OVX+v using the Steel test.

(C) Quantity of food intake for 24 hours at 2 weeks after the operation. *p < 0.05 using the Steel test.
Figure 2A, B. OVX+GSE mice showed significantly lower blood glucose levels at 30 min and 60 min than those in OVX+V mice (Figure 2B). These data suggest that GSE administration improved glucose tolerance in OVX mice.

Discussion

We demonstrated the anti-obesity effects of GSE in OVX mice. Although food intake was enhanced by OVX, as reported previously, it was not affected by GSE administration. These results indicate that the anti-obesity effect of GSE may be independent of appetite regulation. In addition to reduced adipose tissues, glucose tolerance was improved by GSE in OVX mice, consistent with the relationship between increase visceral fat depots and aberrant glucose tolerance.

It is not yet known whether reduction in adipose tissues and improved glucose tolerance result from higher physical activity or enhanced energy expenditure. As UCP-1 expression regulates body fat mass, UCP-1 expression in WAT and BAT was evaluated in sham,
GSE is prevalent in the colon owing to its poor absorption ability. The gut microbiota contributes to metabolic disorders, such as obesity and glucose intolerance. Low diversity in gut communities is associated with obesity in humans and rodents. Metagenomic and biochemical analyses have indicated that obesity is associated with changes in the relative abundance of the two dominant bacterial phyla, Bacteroidetes and Firmicutes. A metagenomic study indicated that several gut bacterial species are linked to metabolic risk markers in postmenopausal women with obesity. In rodents, GSE administration prevents high-fat-diet-induced obesity, improves metabolic parameters, and upregulates fatty acid oxidation-related genes in the liver. The gut microbiota is altered by a high-fat diet, and GSE administration is reported to ameliorate high-fat diet-induced obesity by targeting the gut microbiota. Recently, we found that the increased population of Firmicutes and decreased population of Bacteroidetes induced by ovariectomy recover to normal levels after the oral intake of GSE in an OVX mouse model (in review). Combined with the close relationship between obesity and the ratio of Firmicutes: Bacteroidetes populations, proanthocyanidin-rich GSE is expected to contribute to the improvement of obesity in postmenopausal women via the gut microbiota. In conclusion, we demonstrated that GSE prevents metabolic abnormalities in OVX mice. These results support the potential therapeutic applications of GSE in postmenopausal women.

References


