Brazilian Green Propolis Promotes Weight Loss and Reduces Fat Accumulation in C57BL/6 Mice Fed A High-Fat Diet

Tohru Sakai,* Miyuki Ohhata, Misaki Fujii, Sayaka Oda, Yasuna Kusaka, Miki Matsumoto, Akiko Nakamoto, Tomoyo Taki, Mariko Nakamoto, and Emi Shuto

Department of Public Health and Applied Nutrition, Institute of Biomedical Science, Tokushima University of Graduate School; 3–18–13 Kuramoto-cho, Tokushima 770–8503, Japan.

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Propolis is a bee product with various biological properties. C57BL/6 mice were fed a high-fat diet and treated with propolis for 14 weeks. Body weight in mice treated with 2% propolis was less than that in control mice from 3 weeks after the start of treatment until 14 weeks except for the 7th week. Mice treated with propolis showed significantly lower epididymal fat weight and subcutaneous fat weight. Infiltration of epididymal fat by macrophages and T cells was reduced in the propolis group. Supplementation of propolis increased feces weight and fat content in feces, suggesting that mechanisms of weight reduction by propolis partly include a laxative effect and inhibition of fat absorption.

Key words propolis; weight; fat absorption

The incidence of metabolic syndrome is increasing worldwide because of the increasing number of people with a sedentary lifestyle and people who overeat. Metabolic syndrome is associated with increased risk of cardiovascular diseases and type II diabetes. Obesity is the fundamental factor for the development of metabolic syndrome. In the United States, obesity is responsible for approximately 300000 deaths per year. Individuals who are obese are susceptible to infections and more likely to develop serious complications from common infections. Therefore, much attention has been paid to the development of functional food for weight control.

Propolis is a resinous substance collected by Apis mellifera from various tree buds. Much interest has recently been shown in medical applications of propolis because it contains many types of polyphenol. Recent studies have shown that propolis possesses cytotoxic, antiherpes, free radical scavenging, antimicrobial and anti-human immunodeficiency virus (HIV) activities.4–8)

In this study, we examined the effect of propolis on weight control in mice fed a high-fat diet and investigated the mechanism by which propolis suppresses weight gain.

MATERIALS AND METHODS

**Mice and Diet** Male C57BL/6J mice (Japan SLC, Shizuoka, Japan) were maintained under specific pathogen-free conditions with a 12-h light : dark cycle at 25±2°C and 55±10% relative humidity. The composition of food was 27.5% casein, 17.6% α-starch (Oriental Yeast Co., Ltd., Chiba, Japan), 8.3% sucrose (Mitsui Sugar Co., Ltd., Osaka, Japan), 36.0% lard (Oriental Yeast Co.), 2% cellulose, 5% mineral mixture, 1% vitamin mixture (Oriental Yeast Co., Ltd.), 0.3% dl-methionine (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 0.2% choline bitartrate (Wako) and 2% dextrin (Wako). Brazilian propolis was supplied as a powder by Yamada Bee Farm (Okayama, Japan). Brazilian propolis is produced in Southeast Brazil, and Baccharis dracunculifolia is the main botanical source. It contains mainly prenylated derivatives of cinnamic acid including artepillin C as a major component (6.1%). In the experimental diet, propolis was added to the control diet at a dose of 2.0% (w/w) instead of dextrin. All experimental procedures were approved by the Animal Research Committee of the University of Tokushima.

**Determination of Body Fat Percentage** The percentage of body fat at 12 weeks after starting propolis treatment was measured by X-ray computed tomography (CT; LaTheta; Aloka, Tokyo, Japan) from the first lumbar vertebra to the pubic bone, under isoflurane anesthesia. Data were analyzed using LaTheta software.

**Indirect Calorimetry** Four-week-old male C57BL/6J mice were given the high-fat diet supplemented with or not supplemented with 2% propolis for 4 weeks because a significant difference in body weight was observed from 3 weeks after the start of propolis treatment. Oxygen consumption was continuously measured during the 12-h light-dark cycles using a comprehensive laboratory animal open-circuit indirect calorimetry monitoring system (Columbus Instruments, Columbus, OH, U.S.A.).

**Flow Cytometry** For analysis of the macrophage population in adipose tissue, stromal vascular cells were stained with phycoerythrin (PE)-conjugated anti-F4/80 monoclonal (m) antibody (Ab) and fluorescein isothiocyanate (FITC)-conjugated CD45 monoclonal antibody (mAb). For analysis of the T cell population in adipose tissue, stromal vascular fraction cells were stained with PE-conjugated CD3 mAb and FITC-conjugated CD45 mAb. All Abs were purchased from eBioscience (CA, U.S.A.). Flow cytometric analysis was performed on Guava easyCyte using Guava Express Pro software (Merck Millipore, Darmstadt, Germany).

**Determination of Lipid Content in Feces** Feces were collected from control mice and propolis-treated mice from 1 to 4 weeks after the start of treatment because a significant difference in body weight was observed from 3 weeks after the start of propolis treatment. Collection was done twice per week. The amount of lipid in feces was determined according to the Folch method with some modification. The feces were homogenized with 10 mL of water. Two mL of the homogenate was mixed with 5 mL of chloroform–methanol (2:1). After

* To whom correspondence should be addressed. e-mail: sakai@tokushima-u.ac.jp

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vigorously shaking the sample, the tube was centrifuged at 3000 rpm for 5 min. The lower chloroform phase was collected, and chloroform/methanol was added to the residual sample and centrifuged at 3000 rpm for 5 min. The lower phase was collected again. The lower chloroform phase mixture containing lipids was filtrated and dried at 80°C to vaporize the solvents, and then the lipid content was measured.

**Lipid Contents**  Total cholesterol and triglycerides (TG) in serum, the liver and feces were measured by using enzymatic kits (Cholesterol E test Wako and Triglyceride E test Wako; Wako Pure Chemical Industries, Ltd.).

**Statistics** Data are shown as means and standard deviation. Statistical significance in the difference between two means was assessed by the unpaired t-test and Mann–Whitney U-test. p Values <0.05 were considered to be statistically significant.

**RESULTS**

**Propolis Attenuated Gain of Body Weight in Mice Fed a High-Fat Diet** Mice were fed a high-fat diet during the experimental period. Both mice fed a high-fat diet and those fed a high-fat diet plus propolis showed a day-by-day increase in body weight. Body weight in propolis-treated mice was less than that in control mice from 3 weeks after the start of the experiment. A reduction in body weight gain in the...
propolis group was observed from 3 to 14 weeks except for the 7th week (Fig. 1). Although we determined food intake in both groups, no significant difference was observed between the two groups (control group, 3.2±0.2 g/d; propolis group, 3.3±0.4 g/d). Therefore, decrease in food intake in the propolis group was not the cause of change in body weight. In addition to mice fed the high-fat diet, we observed a gain of body weight in mice fed a normal chow diet and those fed a normal chow plus 2% propolis diet, but a significant difference in body weight was not observed between the control and propolis groups (data not shown).

**Propolis Suppressed Body Fat Accumulation** Body fat was weighed and the weights in the control group and propolis group were compared. As shown in Figs. 2A and B, both epididymal fat weight and subcutaneous fat weight in mice treated with propolis were significantly lower than those in control mice. CT scan analysis showed that the percentage of body fat in the propolis group was also lower than that in the control group (Fig. 2C).

**Propolis Suppressed Infiltration of Epididymal Fat by Immune Cells** Stromal vascular fraction cells were prepared and the percentages of T cells and macrophages were determined by flow cytometric analysis. In this study, we defined F4/80<sup>+</sup> cells and CD3<sup>+</sup> cells as macrophages and T cells, respectively. The percentages of F4/80<sup>+</sup> cells and CD3<sup>+</sup> cells in mice treated with propolis were lower than those in control mice (Fig. 3).

**Treatment with Propolis Did Not Change Energy Expenditure** Treatment with propolis reduced the gain of body weight and fat accumulation in mice fed a high-fat diet. To elucidate the mechanism, we determined energy expenditure by indirect calorimetry. Oxygen consumption during the light and dark cycles was not changed by propolis treatment (Fig. 4).

**Propolis Increased Feces Weight and Inhibited Fat Absorption** We evaluated feces weight because there is a possibility that propolis decreases body weight by increasing feces weight. Feces weight in the propolis group was significantly greater than that in the control group (control, 262±49 mg; propolis, 326±58 mg, p<0.05) (Fig. 5A). We measured the fat content in feces to determine how propolis affects fat absorption. Fat content in feces from propolis-treated mice was greater than that in feces from control mice (Fig. 5B). We also determined total cholesterol and TG contents in feces, but a difference was not observed between the two groups (Table 1).

**DISCUSSION**

In this study, we found that treatment with propolis reduces body fat content and that the reduction in body fat content may be attributable to increment of feces weight and inhibition of fat absorption (Fig. 5). Previous studies showed that propolis improves insulin sensitivity during the prediabetic and diabetic stages in obese animals. Our results showing that propolis reduces fat accumulation are consistent with results obtained by Ichī et al. Ichī et al. reported that supplementation of propolis reduced white adipose tissue weight and serum levels of cholesterol and TG. As a possible mechanism, they proposed that propolis inhibits TG absorption that results in a reduction of serum TG concentration. In another study by Koya-Miyata, it was shown that treatment with propolis for 10d reduced body weight gain, weight of visceral adipose
Recent studies have shown a crucial role of immune cells of adipose tissue in systemic chronic inflammation and development of metabolic syndrome. It has been shown that immune cells including CD4+ T cells, CD8+ T cells, regulatory T cells, and eosinophils contribute to the differentiation of inflammatory macrophages. Kitamura proposed that Brazilian propolis improves blood glucose and plasma cholesterol levels via its effect on immune cells of adipose tissue. Propolis promotes the proliferation of eosinophils and suppresses the proliferation of inflammatory macrophages in adipose tissue. We analyzed the immune cell population in adipose tissue and found reduced percentages of T cells and macrophages (Fig. 3). Different from the results reported by Kitamura, we did not observe an improvement of blood glucose levels after oral glucose and insulin administration (data not shown). It is debatable whether infiltration of adipose tissue by immune cells is an essential point for the weight reduction action of propolis. We speculate that reduced infiltration of immune cells in adipose tissue might result from reduced fat accumulation and/or attenuation of gain of body weight in propolis-treated mice.

We determined energy expenditure by indirect calorimetry. Oxygen consumption during the light and dark cycles was not changed by propolis treatment (Fig. 4), indicating that propolis does not directly enhance energy expenditure. We found that the weight and fat content of feces from propolis-treated mice were increased compared to those of feces from control mice (Fig. 5). Treatment with the water extract, but not ethanol extract, of propolis significantly increased stool weight and significantly restored stool frequency in a clonidine-induced constipation model. To our knowledge, this is the first study showing that ethanol-extracted propolis suppresses weight gain by increasing feces weight and inhibiting fat absorption. Propolis contains a variety of chemical compounds including polyphenols, flavonoids, phenolic aldehydes, amino acids, and vitamins. The mechanism of the action of propolis on physiological metabolism including weight loss has been investigated in some studies. Ferulic acid has been shown to promote weight loss in rats and improve serum glucose levels in mice fed a high-fat diet. Intake of kaempferol ameliorates hyperglycemia, hyperinsulinemia and circulating lipid profile, which are associated with improved peripheral insulin sensitivity in mice fed a high-fat diet. Upregulation of carnitine palmitoyltransferase 1 by p-coumaric acid improves liver fat metabolism. Further studies are needed to clarify what components are effective for weight control.

Table 1. Effects of Propolis on Lipids in Serum, the Liver and Feces

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<thead>
<tr>
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<th>Control</th>
<th>Propolis</th>
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<tr>
<td>Serum (mg/dL)</td>
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<tr>
<td>Cholesterol</td>
<td>328.2±15.7</td>
<td>345.6±15.7</td>
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<tr>
<td>TG</td>
<td>342.2±24.1</td>
<td>335.9±17.2</td>
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<tr>
<td>Liver (mg/g)</td>
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<td></td>
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<tr>
<td>Cholesterol</td>
<td>3.37±0.38</td>
<td>3.96±0.83</td>
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<tr>
<td>TG</td>
<td>108.8±33.2</td>
<td>102.9±25.9</td>
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<tr>
<td>Feces (mg/g)</td>
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<tr>
<td>Cholesterol</td>
<td>0.92±0.38</td>
<td>0.73±0.27</td>
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<tr>
<td>TG</td>
<td>2.47±0.82</td>
<td>1.92±0.65</td>
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Data are presented as means±S.D. of 8 mice for serum and liver data and as the mean±S.D. of 7 determination for feces data.

In conclusion, we found that Brazilian propolis promotes weight loss and suppresses infiltration of adipose tissue by immune cells. Increased feces weight and inhibition of fat absorption are mechanisms of weight loss.
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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


