Propolis Prevents Diet-Induced Hyperlipidemia and Mitigates Weight Gain in Diet-Induced Obesity in Mice

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We examined the hypolipidemic effect of propolis in a mouse obesity model induced by a high fat-diet. C57BL/6N mice were fed a high-fat diet ad libitum and given propolis extract intragastrically at 0 mg/kg (control), 5 mg/kg or 50 mg/kg twice daily for 10 d. Compared with mice in the control group, mice in the propolis extract-administered groups showed a reduction in all of the following parameters: body weight gain, weight of visceral adipose tissue, liver and serum triglycerides, cholesterol, and non-esterified fatty acids. Real-time polymerase chain reaction analysis of the liver showed down-regulation of mRNA expression associated with fatty acid biosynthesis, including fatty acid synthase, acetyl-CoA carboxylase α, and sterol regulatory element binding protein in the propolis-administrated mice. Subsequently, obese C57BL/6N mice that had been administered a high-fat diet were given propolis extract at 0 mg/kg (control), 2.5 mg/kg or 25 mg/kg for 4 weeks. The propolis extract treated mice showed a decrease in weight gain, a reduction of serum non-esterified fatty acids, and lipid accumulation in the liver. These results suggest that propolis extract prevented and mitigated high-fat diet-induced hyperlipidemia by down-regulating the expression of genes associated with lipid metabolism.

Key words propolis; visceral adipose tissue; triglyceride; fatty acid biosynthesis

The accumulation of visceral adipose tissue usually results from an energy imbalance involving the combination of excessive calorie consumption with insufficient energy expenditure. Increased visceral adiposity can induce insulin resistance and lead to type 2 diabetes mellitus, dyslipidemia, hypertension, and cardiovascular disease.1 “Metabolic syndrome” is the combination of multiple cardiovascular risk factors, including visceral obesity, dyslipidemia, glucose intolerance, and hypertension in one individual.2,3 Obesity resulting from an accumulation of visceral adiposity is a primary cause of metabolic syndrome.

Propolis is a sticky, resinous substance collected by honey bees (Apis mellifera) from the sap, leaves, and buds of plants, and then mixed with secreted beeswax. The chemical constituents of propolis are mainly flavonoids, phenolic compounds, caffeoylquinic acids, cinnamic acid derivatives, diterpenoic acid, lignan, coumarin acid and other compounds.4,5 Propolis has been used as a folk medicine in many countries from ancient times especially in Brazil and Eastern Europe. It has been characterized variously as an anti-bacterial,6,7 anti-viral,8 anti-inflammatory,9–11 anti-oxidant,12 and anti-carcinogenesis agent.13,14 Obesity is caused by various environmental and genetic factors.15 One of the main environmental factors causing obesity is the intake of a high-fat diet, now common in many populations. A diet-induced obesity animal model has been developed to investigate human obesity, replicating the effects of human obesity more accurately than genetic obesity models.16 Mice that are fed a high-fat diet, develop obesity, hyperglycemia, and hyperlipidemia, and differ from those fed a normal diet in the expression levels of specific genes transcripts.17 Recent studies have reported that propolis prevented and mitigated diabetes and hypertension,18–22 but it remains unclear whether propolis similarly prevents and mitigates the accumulation of visceral adipose tissues and hyperlipidemia. In this study we examined the effect of propolis on visceral fat and hyperlipidemia induced in a high-fat diet-induced obesity mouse model, and analyzed hepatic gene expression involved in lipid metabolism.

MATERIALS AND METHODS

Preparation of Propolis Extract Propolis used in this study was obtained from Apiai, Sao Paulo in Brazil. Propolis (1 kg) was treated with 50% ethanol (3 l) at room temperature for 24 h, and filtered. This extract included 10.8% of solid contents of propolis. Total flavonoids content in the extract was determined as amounts of quercetin by using the aluminum nitrate method as described previously.23 In addition, the extract was subjected to reversed-phase column chromatography using following conditions: column, Shim Pack CLC-ODS (6 mm i.d. × 150 mm, Shimadzu, Japan); column temperature, 50 °C; gradient system, A solvent (30% MeOH, 70% H2O and 1% AcOH) and B solvent (75% MeOH, 25% H2O and 1% AcOH), 0—20 min 100% A isocratic, 20—55 min 100% B isocratic, 55—75 min 100% A isocratic; flow rate, 0.8 ml/min; detection, UV 275 nm. As described previously, 3,5-diprenyl-4-hydroxycinnamic acid (artepillin C) and 3-prenyl-4-hydroxyacinnamic acid (drupinin C) were purified from the propolis extract and identified by NMR spectroscopy at our institute.24 Purified preparations of artempillin C (purity 99.0%) and drupinin C (purity 98%) were used as standards. HPLC analysis of the extract showed a characteristic profile of Brazilian propolis, and cinnamic acid derivatives such as artempillin C and drupinin C, which are distinctively contained in Brazilian propolis, were detected.25 The extract used in this study contained 7.6 mg/ml of flavonoids, 8.6 mg/ml of artempillin C and 3.7 mg/ml of drupinin C.

Animals Six-week-old C57BL/6N mice were obtained from Charles River Japan, and fed a standard diet and water ad libitum for 1 week. The mice were kept in a temperature controlled room with a 12-h light cycle. This study was approved by the Laboratory Animal Care Committee of Hayashibara Biochemical Laboratory, and all animal experi-
ments were conducted in accordance with the Guidelines of Care and Use of Laboratory Animals at the Hayashibara Biochemical Laboratory.

**Obesity Prevention Experiment** Seven-week-old male mice were randomly divided into 3 groups (8 to 9 mice per group) and matched for body weight. A modified AIN-93G diet with a high fat content (50% of crude fat) was used as the basal diet. Its dietary constituents per 100 g food were as follows: bean oil 25 g, casein 25 g, alpha-corn starch 6.2 g, beta-corn starch 18.4 g, sugar 13 g, cellulose 6.3 g, vitamin mixture (AIN-93G) 1.3 g, mineral mixture (AIN-93G) 4.4 g, l-cystine 0.4 g, and t-butylhydroquinone 0.0014 g. The mice were administered either the vehicle alone (2% EtOH) or with propolis at doses of 5 mg/kg or 50 mg/kg by stomach intubation twice daily for a period of 10 d. All samples were administered in a volume of 200 μl.

**Adipose Tissues, Serum and Liver Analysis** On day 11 following overnight fasting, the mice were euthanized under ether anesthesia. Blood samples were collected from the abdominal aorta to measure serum triglycerides, total cholesterol, non-esterified fatty acid, and glucose as determined by enzymatic colorimetric test kits (triglyceride E-test, cholesterol E-test, NEFA C-test, and glucose CII-test) from Wako Pure Chemical Industries (Tokyo, Japan). Adipose tissues and liver were removed and weighed. Liver lipids were extracted by the procedure of Folch et al.27

**RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (PCR)** Total RNA was extracted from each liver sample using the RNeasy Mini kit (QIAGEN, Tokyo, Japan) and DNase (QIAGEN) according to the manufacturer’s instructions. Subsequently, first-strand cDNA was synthesized using superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, U.S.A.) and Oligo(dT)12–18 (Invitrogen). Specific primers for PCR were either identical to those described previously17) or were designed using Primer3, and real-time PCR was performed on LightCycler 480 system (Roche). For quantitative precision, the expression amounts of each gene were normalized to the housekeeping gene, Cyclophilin A (CYP A). Change in gene expression by administration of propolis was estimated as fold change relative to the mean value of control mice.

**Measurement of Lipid Metabolism-Related Enzyme Activities in the Liver** The frozen liver was homogenized in 3 mm Tris HCl buffer (pH 7.2) containing 0.25 m sucrose and 1 m ethylenediaminetetraacetic acid (EDTA). The homogenate was then centrifuged for 10 min at 500 g, and the supernatant was removed and centrifuged for an additional 10 min at 9000 g. This supernatant was used for the assay of fatty acid synthase (FAS) enzymes. FAS enzyme activity was measured as described previously.28) The enzyme activities were measured by the spectrophotometric assay using a Shimadzu spectrophotometer model UV2400PC (Shimadzu, Kyoto, Japan). Protein concentrations of the homogenates were measured by the Bradford method29) with a protein assay kit (Bio-Rad, Richmond, CA, U.S.A.) using bovine serum albumin as a standard.

**Pre-existing Obesity Mitigation Experiment** A total of 24 mice were used in this study. Seven-week-old female mice were fed a commercial diet High Fat Diet 32 (Clear, Japan) to induce accumulation of visceral adipose tissues and insulin resistance for 8 weeks, and were then divided into 3 groups of 8 mice per group that were matched for body weight. The mice were then administered either the vehicle alone (2% EtOH) or with propolis extract at doses of 2.5 mg/kg or 25 mg/kg by stomach intubation twice daily continuously for 4 weeks (5 d per week). The mice were fed with High Fat Diet 32 and water ad libitum during the 4-weeks experimental period. On day 26, following overnight fasting, the mice were euthanized under ether anesthesia. Adipose tissues were weighed, and blood samples were collected to measure serum glucose and lipids.

**Statistics** Statistical analysis was performed using the JMP program (version 6.0) and all measurements were expressed as mean±S.D. Statistically significant differences between the groups were identified using Dunnett’s test.

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**Table 1. Sequences of Primers for the Real-Time Polymerase Chain Reaction Analysis**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward</th>
<th>Reverse</th>
<th>GenBank Accession No.</th>
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</thead>
<tbody>
<tr>
<td>SREBP-1 251</td>
<td>GGCACATTAGTCGCCCTAACCCT</td>
<td>GCCCATAGATCTCTGCAGGATGTC</td>
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</tr>
<tr>
<td>SREBP-2</td>
<td>GAAGTTGACGGAGGTCCTCT</td>
<td>GCGCATAGTGACAGGCAGACAG</td>
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</tr>
<tr>
<td>PPARα</td>
<td>ATGCCAGTCTGCGGTTC</td>
<td>GTATGACAAAGGCCGTGTTT</td>
<td>NM_011144</td>
</tr>
<tr>
<td>ALCY</td>
<td>AGAAGTGGCTAGAGTTGAAAC</td>
<td>CTGACCTTCAAGATGAGGAT</td>
<td>NM_134037</td>
</tr>
<tr>
<td>ACAC</td>
<td>GAGGGGTTCAAGTCCTCTT</td>
<td>ACATCACCCTTCCACACAGC</td>
<td>NM_133360</td>
</tr>
<tr>
<td>MCAT</td>
<td>TGAAGGTCTGAGTGCGTGA</td>
<td>GAGATGACCCTGATCAG</td>
<td>NM_01030014</td>
</tr>
<tr>
<td>FAS</td>
<td>TTGCATCATCGTACAGATGC</td>
<td>TTACACCTTGTCCTTCTGCT</td>
<td>NM_007988</td>
</tr>
<tr>
<td>HMGCS1</td>
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<tr>
<td>HMGCR</td>
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</tr>
<tr>
<td>SQLE</td>
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<td>TCCTAGTTGACGTTGTA</td>
<td>NM_009270</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>GATCAGGACTGCTGAAAGCA</td>
<td>GCTGTCGCGATATCAGGAA</td>
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</tr>
<tr>
<td>CYP A</td>
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<td>GATGGAAGGCTGCGGTATGCT</td>
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</table>

Specific primers for each gene were designed using Primer3 or were used as described previously.17) SREBP-1: sterol regulatory element binding protein-1, SREBP-2: sterol regulatory element binding protein-2, PPARα: peroxisome proliferator activated receptor α, ALCY: ATP citrate lyase, ACAC: acetyl-CoA carboxylase α, MCAT: malonyl CoA:ACP acyltransferase, FAS: fatty acid synthase, HMGCS1: 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1, HMGCR: 3-hydroxy-3-methylglutaryl-Coenzyme A reductase, SQLE: squalene epoxidase, CYP7A1: cholesterol 7α-hydroxylase, CYP A: cyclophilin A (housekeeping).
RESULTS

Obesity Prevention Experiment Figure 1 shows the weight gain of each mouse group during the experimental period. Administration of 50 mg/kg propolis extract significantly reduced the weight gain compared to the control group. Food intake during the experimental period was not affected by propolis extract, since daily food intake in mice of control and those of administrated 5 mg/kg and 50 mg/kg propolis extract was 2.71±0.31, 2.64±0.31, and 2.73±0.31 g/d/body, respectively. These results suggest that propolis is a pharmacological inhibitor of weight gain.

Since propolis extract reduced weight gain, we examined its effect on the liver and the adipose tissue weights in high-fat diet-fed obese mice (Fig. 2). The retroperitoneal adipose tissue weights in mice administered 5 mg/kg and 50 mg/kg propolis extract were 0.20±0.07 and 0.21±0.06 g, respectively, and both of these were significantly lower than the tissue weight of the control group (0.26±0.05 g). Liver, perirenal and epididymal adipose tissue weights all tended to be lower than those of the control group, although the differences were not statistically significant. In the control group, the total weight of visceral adipose tissues (including retroperitoneal, perirenal and epididymal adipose tissues) was 0.90±0.15 g. Those in the propolis extract 5 mg/kg and 50 mg/kg groups were 0.73±0.17 and 0.72±0.19 g, respectively. A significant difference between the propolis extract 50 mg/kg group and the control group was observed in the total visceral adipose weight.

Table 2. Effect of Propolis Extract on Serum Compounds and Liver Triglycerides in Male Mice Fed a High-Fat Diet for 10 d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Propolis extract 5 mg/kg</th>
<th>Propolis extract 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>102.5±33.1</td>
<td>75.6±12.5*</td>
<td>88.3±16.3</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>117.0±13.0</td>
<td>106.2±6.7*</td>
<td>118.3±7.0</td>
</tr>
<tr>
<td>Non-esterified fatty acid (mEq/l)</td>
<td>0.83±0.10</td>
<td>0.73±0.08*</td>
<td>0.75±0.06*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>141.2±13.9</td>
<td>136.0±26.4</td>
<td>145.0±10.5</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.331±0.159</td>
<td>0.188±0.168</td>
<td>0.188±0.074</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/g)</td>
<td>7.34±1.37</td>
<td>6.06±0.76*</td>
<td>5.32±0.44**</td>
</tr>
</tbody>
</table>

Data are presented as the mean±S.D. of 8—9 mice. * Indicates a p-value <0.05 (versus the control group). ** Indicates a p-value <0.01 (versus the control group).

The effect of propolis extract on serum and liver lipid parameters of mice fed a high-fat diet for 10 d was examined (Table 2). Serum triglycerides and cholesterol levels were significantly lower in the propolis extract 5 mg/kg group compared to levels in the control group. Serum non-esterified fatty acid levels were significantly lower in the propolis extract 5 mg/kg and 50 mg/kg group than those in the control group. Serum glucose levels in the propolis-treated groups were comparable with those in the control group, but serum insulin levels tended to be lower than those of the control group (p=0.09 and p=0.09 for 5 mg/kg and 50 mg/kg propolis extract group, respectively). In addition, hepatic triglyceride levels were significantly reduced in a dose-dependent manner by the administration of propolis compared with those in the control group.

Lipid Metabolism-Related Genes and Enzyme Activity of the Liver To examine whether administration of propolis extract has a beneficial effect on lipid metabolism in vivo, the expression of genes involved in lipid metabolism was measured in liver using real-time PCR. We first analyzed mRNA levels of transcription factors associated with lipid metabolism (Fig. 3A). Expression levels of sterol regulatory element binding protein-1 (SREBP-1), a key factor in transcriptional regulation of fatty acid synthesis, were significantly reduced by about 30% by administration of propolis extract (5 mg/kg, 50 mg/kg). The higher dosage of propolis extract also down-regulated mRNA levels of sterol regulatory element binding protein-2 (SREBP-2), which controls cholesterol biosynthesis. In contrast, no difference was observed in the mRNA levels of peroxisome proliferators-activated re-

Fig. 1. Effect of Propolis on Mouse Weight Gain

C57BL/6N male mice were fed a high-fat diet and given either vehicle alone (2% ethanol), or with 5 mg/kg or 50 mg/kg propolis extract per day for 10 d. Body weight for each mouse was measured every 3 or 4 d. * Indicates p-value <0.05 (versus the control group).

Fig. 2. Liver and Visceral Adipose Tissue Weights in High-Fat Diet Fed Mice That Were Given Propolis Extract for 10 d

Total visceral weight indicates the sum of retroperitoneal, epididymal, and perirenal adipose tissues. * Indicates p-value <0.05 (versus the control group).
ceptor \(\alpha\) (PPAR\(\alpha\)).

We next examined the gene expression of several fatty acid synthesis enzymes (Fig. 3B). Expression levels of genes associated with transcription factors (A), fatty acid synthesis (B), and cholesterol metabolism (C) were analyzed by real-time PCR. Levels of Cyclophilin A (CYP\(A\)) mRNA were used for normalization. Changes in gene expression resulting from the administration of propolis were expressed as the fold changes relative to the mean values of control group. Values represent the mean \pm S.D. of 8—9 mice. * Indicates \(p\)-value <0.05 (compared with the control group), ** indicates \(p\)-value <0.01 (compared with the control group).

Fig. 3. Hepatic Gene Expression Analysis

Total RNA was extracted from liver samples, and subsequently first-strand cDNA was synthesized using superscript III reverse transcriptase as described. Expression levels of genes associated with transcription factors (A), fatty acid synthesis (B), and cholesterol metabolism (C) were analyzed by real-time PCR. Levels of Cyclophilin A (CYP\(A\)) mRNA were used for normalization. Changes in gene expression resulting from the administration of propolis were expressed as the fold changes relative to the mean values of control group. Values represent the mean \pm S.D. of 8—9 mice. * Indicates \(p\)-value <0.05 (compared with the control group), ** indicates \(p\)-value <0.01 (compared with the control group).

Fig. 4. FAS Enzyme Activity in Liver

FAS enzyme was extracted from liver samples, and the activity was measured as described. Data are presented as the mean \pm S.D. of 8—9 mice. ** Indicates a \(p\)-value <0.01 compared with the control group.

Fig. 5. Effect of Propolis Extract on Weight Gain in Obese Mice

High-fat diet-induced obese C57BL/6N female mice were given either the vehicle alone (2% ethanol, circle), or with 2.5 mg/kg (box) or 25 mg/kg propolis extract (triangle) for 26 d. * Indicates a \(p\)-value <0.05 compared with the control group.

Pre-existing Obesity Mitigation Experiment

Since propolis extract prevented the high-fat diet-induced accumulation of visceral adipose tissues and hyperlipidemia in young adult mice, we further examined the effect of propolis extract in obese mice that had been induced by a high-fat diet for 8 weeks. This corresponded to a 200% body weight gain over an 8-week period. The average body weight of 24 mice was 38.9 ± 2.8 g at the beginning of the experiment. Propolis extract (2.5 mg/kg, 25 mg/kg) was then given to the obese mice by stomach intubation twice daily for 4 weeks. Figure 5 shows the body weight gain in each group of mice during the 4-week experimental period. Administration of 25 mg/kg propolis extract significantly reduced the body weight gain compared with the control group after 7 d. This weight gain was reduced by the administration of 2.5 mg/kg propolis ex-
tract, although the differences were not statistically significant. Daily food intake in mice of control and those of administered 2.5 mg/kg and 25 mg/kg propolis extract was 1.91, 1.93, and 1.86 g/d/subject, respectively, suggesting that food intake during the experimental period was not affected by propolis administration.

The effects of propolis extract on adipose tissue weights, serum parameters and liver lipids was examined, and the results are shown in Table 3. The perirenal adipose tissue weight in obese mice administered 25 mg/kg propolis extract was significantly lower than that of the control group. Changes in the retroperitoneal and parametrial adipose tissue weights were not statistically significant. The hepatic triglyceride levels were significantly reduced by administration of propolis extract compared with those in the control group. Similarly, serum triglyceride levels tended to be lower in the propolis groups. The serum non-esterified fatty acid levels were significantly lower in the propolis extract 2.5 mg/kg and 25 mg/kg group than those in the control group. The glucose levels were significantly lower in the propolis extract 25 mg/kg group than those in the control group, and the insulin levels in the propolis extract groups tended to be lower than those of the control group ($p=0.06$ and $p=0.07$ for 2.5 mg/kg and 25 mg/kg propolis extract, respectively).

**DISCUSSION**

The prevalence of obesity is increasing worldwide. It is generally accepted that consumption of a high-fat diet contributes directly to visceral obesity in both humans and animals. Furthermore, visceral obesity induced by a high-fat diet is associated with increased risk for chronic diseases, including cardiovascular disease, certain types of cancer, diabetes, hyperlipidemia, and hypertension. These findings suggest the importance of preventing this accumulation of visceral obesity for the promotion of human health.

Recent studies reported that propolis could prevent and/or mitigate diabetes and hypertension, but it is not known whether propolis also reduces the accumulation of visceral adipose tissues and hyperlipidemia. The present study was conducted to test whether propolis could reduce hyperlipidemia in a mouse model. In the first experiment we examined the effects of propolis extract on the elevation of visceral adipose tissue weight and serum and liver triglyceride levels in mice fed a high-fat diet over a short-term period. In the second experiment, propolis extract was administered to obese mice that had been fed a high-fat diet for 4 weeks, and changes in weight gain and lipids in serum and liver were evaluated.

In the first experiment, propolis extract inhibited both weight gain (Fig. 1) and the increase in visceral adipose tissue weight (Fig. 2). In addition, propolis extract reduced the levels of serum triglycerides, cholesterol, non-esterified fatty acids, and liver triglycerides (Table 2). Because propolis prevented high-fat diet-induced obesity, a second experiment was designed to determine whether it also mitigates pre-existing obesity. Propolis inhibited the elevation in the body weight gain (Fig. 5), and reduced the levels of serum non-esterified fatty acid and liver triglycerides (Table 3). In addition, propolis significantly reduced the levels of serum glucose and tended to reduce insulin levels.

We have not measured the locomotor activity of mice administered propolis extract in our experiments in detail, although the momentum is considered to be closely related to the calorie consumption. However, macroscopic observation suggested that there are no differences in the locomotor activity between control mice and propolis extract-administered mice. In accordance with our observations, Kleinrok et al. reported that propolis ethanol extract applied in doses ranging from 10—100 mg/kg in mice did not have an effect on spontaneous movement in these animals.

It has been known that the rise in blood lipids causes obesity, insulin resistance, and that serum lipid levels are affected by diet and lipid-regulating organs such as liver, adipose tissue, and muscle. Furthermore, liver is the most important organ that senses blood glucose, insulin, and lipid, and consistently controls their levels through biosynthesis and catabolism. To clarify the mechanism for the anti-obesity activity of propolis, we focused on hepatic lipid metabolism-related gene expression. The regulation of lipid metabolism in liver is controlled by several kinds of transcription factors, such as SREBP1 and PPARα. SREBP-1 preferentially activates genes involved in fatty acid synthesis, while SREBP-2 preferentially activates genes involved in cholesterol biosyn-
PPARα regulates transcription of genes associated with fatty acid degradation. In our experiment, propolis significantly reduced both SREBP-1 and SREBP-2 mRNA levels. We also found out that the gene expression of FAS and ACAC activated by SREBP-1 was significantly decreased by administration of propolis extract. In addition, the activities of the FAS enzyme in the propolis extract groups were significantly lower than those in the control group. These results suggested that the gene expression involved in fatty acid synthesis correlated strongly with the lipid contents of liver. In contrast, PPARα mRNA levels in the propolis extract groups were similar to those in the control group. Although further studies are needed, we infer that propolis extract had hardly affected the fatty acid degradation via PPARα. These results suggest that one of mechanisms responsible for the inhibitory effect of propolis extract on the accumulation of visceral adipose tissue and hyperlipidemia was down-regulation of fatty acid synthesis.

Next, we examined the gene expression of several downstream targets of SREBP-2. The gene expression of HMGCs and SQLE, encoding early stage enzyme and late stage enzyme of cholesterol biosynthesis, respectively, tended to be down-regulated in the propolis extract groups, but that of HMGCR as key rate-limiting enzyme tended to up-regulate. In addition, the mRNA levels of CYP7A1, one of enzymes associated with bile acid synthesis, were unchanged. Therefore, we failed to clarify the inhibitory mechanism of low-dose of propolis extract on the blood cholesterol in the obesity prevention experiment. However, considering that high-dose propolis extract in the obesity prevention experiment and both low and high doses in the obesity mitigation experiment had no inhibitory effect on the cholesterol level, it seems likely that inhibitory action of propolis on the cholesterol synthesis might not be strong.

Administration of propolis extract tended to decrease serum insulin levels in both the obesity prevention experiment and mitigation experiment. It is known that insulin increases the gene expression of SERBP-1 in liver. Therefore, it is suggested that inhibition of insulin levels in mice administrated propolis extract caused down-regulation of SREBP-1 gene expression, resulting in a decrease of the fatty acid synthesis.

Recent study reported that propolis prevented the development of insulin resistance induced by fructose-drinking rats, and that improvement of hyperglycemia and hypertension by propolis was ascribed to caffeoylquinic acid as one of the major bioactive constituents. Therefore, it may be worthwhile to examine the hyposalipidemic effect of caffeoylquinic acid in our experimental system.

In addition, we found that propolis extract inhibited insulin resistance of differentiated adipocyte in vitro using a the 3T3-L1 preadipocytes cell line. These results suggest that propolis extract may regulate lipid metabolism and improve insulin sensitivity. Further studies of gene expression in other tissues such as adipose tissues and muscle may provide additional key information about the hyposalipidemic effect of propolis.

In conclusion, this study demonstrates that oral administration of propolis extract suppressed overall weight gain in mice, the accumulation of visceral adipose tissue weight, and the increase in serum and liver triglycerides that normally result from feeding a high-fat diet to C57BL/6N mice. Real-time PCR results suggested that the anti-obesity effects of propolis extract could be attributed to reduced expression of fatty acid synthesis genes in the liver. In addition, propolis extract inhibited body weight gain and liver triglycerides in obesity induced by a high-fat diet. Since it is known that accumulation of visceral adipose tissue and hyperlipidemia can induce metabolic syndrome, these results suggest that propolis extract could prevent and mitigate metabolic syndrome caused by excessive intake of a high-fat diet, and that this may occur by down-regulating lipid metabolism-related gene expression.