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Improvement of insulin resistance, blood pressure and interstitial pH in early developmental stage of insulin resistance in OLETF rats by intake of propolis extracts

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ABSTRACT

Propolis, a resinous mixture collected from plants by the *Apis mellifera* bee, contains high level nutrient factors including vitamins, polyphenols, and amino acids that would be expected to improve insulin sensitivity. Insulin resistance would secondarily cause elevation of blood pressure and increase the risk of cardiovascular diseases. The purpose of this study is to investigate the effect of propolis extracts on blood glucose levels and blood pressures in an early developmental stage of insulin resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. OLETF rats (10 weeks old) were divided into three different groups: normal diet, 0.1% propolis diet, and 0.5% propolis diet. After 8 weeks, blood glucose levels, blood pressures, plasma metabolic factors and hormones, and interstitial fluid pH were measured. Casual blood glucose levels were decreased associated with a reduction of plasma insulin levels in both propolis diet groups compared with normal diet group. Propolis decreased systolic blood pressure with no significant changes in plasma aldosterone levels. We also found that interstitial fluid pH in ascites, liver, and skeletal muscle was higher in rats fed propolis diet than rats fed normal diet. These data suggests that dietary propolis improves insulin sensitivity and blood pressures in the early stage of the process in development of insulin resistance, which may be mediated by suppression of metabolic acidosis.

1. Introduction

Common diseases on the basis of metabolic disorders, which are called metabolic syndrome, are developing worldwide. Metabolic syndrome refers to a collection of issues including visceral obesity, elevated blood glucose levels, dyslipidemia (elevated fasting triglycerides and low high-density lipoprotein cholesterol levels), and hypertension. It leads to an increase in the risk of developing of cardiovascular disease, type 2 diabetes, and cancer; therefore, it could be a pre-disease state. Thus, effective strategies, mainly adequate diets, preventing metabolic syndrome are required to decrease the incidence of diseases and promote healthy aging.

It is well known that insulin resistance plays a critical role in development of cardiovascular disease. Cross-sectional and longitudinal cohort studies [1–4] have shown that blood pressure, a typical clinical marker for diagnosis of cardiovascular function, is closely associated with insulin sensitivity and is elevated with development of insulin resistance. Laboratory experiments [5–7] have also suggested that hyperglycemia and hyperinsulinemia cause hypertension via various mechanisms including renal dysfunction, vascular dysfunction, and sympathetic nerve activation. Therefore, preventing the development of insulin resistance could maintain blood pressures at normal levels and consequently prevent metabolic syndrome.

Propolis is a natural product derived from plant resins collected by honeybees and contains many compounds such as polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds [8]. It has been known that propolis possesses anti-microbial, anti-oxidant, anti-inflammation and anti-tumor activities [9–13]. In addition, some compounds contained in propolis might have a potential effect in improvement of insulin sensitivity. Therefore, we hypothesized that ingestion of propolis extract prevents development of insulin resistance and hypertension. In the present study, we investigated the effect of propolis extract on blood glucose levels and blood

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pressures in the early developmental stage of insulin resistance in type 2 diabetic rats.

2. Materials and methods

2.1. Animals and experimental design

All animal studies were performed in accordance with the guidelines of the Japanese Council on Animal Care and approved by the Committee for Animal Research of Kyoto Prefectural University of Medicine. Six-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) rats (SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan) were acclimatized for 4 weeks in a temperature-controlled (22 °C ± 2 °C) room with a 12 h light/dark cycle. The rats were divided into three groups of six, namely, a normal diet group, a 0.1%-propolis diet group, and a 0.5%-propolis diet group. Propolis dried powder of ethanol extracts was prepared from Brazilian propolis (Yamada Bee Farm Inc., Okayama, Japan) and contained to normal diet (AIN-93) at the concentrations of 0.1% w/w and 0.5% w/w, respectively. The rats were fed standard rat chow and had access to water ad libitum. After 8 weeks, systolic blood pressure was measured. The rats were euthanized following measurement of pH, and blood was collected from abdominal aorta for the measurements.

2.2. Blood pressure measurement

The systolic blood pressure was measured under an awaking condition by a tail cuff method (MK-2000, Muromachi-Kikai, Tokyo, Japan).

2.3. pH measurement

The level of pH was measured in ascites and interstitial fluid of skeletal muscle and liver using a microelectrode (Unique Medical Co., Ltd., Tokyo, Japan) under anesthesia. In the pH measurement of skeletal muscle, microprobes were inserted into interstitium between gastrocnemius and tibialis anterior muscles. Urine was collected from bladder, and pH of collected urine was measured using pH indicator tests paper (GE Healthcare Bio-Sciences, Buckinghamshire, UK).

2.4. Blood analysis

Blood glucose concentrations were determined using GluTest (Sanwa Kagaku Co., Ltd., Nagoya, Japan). Immediately after collection, each blood sample was centrifuged at 3500*g* for 15 min at 4 °C. The analysis of the plasma samples was entrusted to FALCO Biosystems Corporation (Kyoto, Japan) conducting measurements of free fatty acid (FFA) and ketone body. For detection of the insulin, enzyme-linked immunosorbent assays were performed using a commercially available kit developed by Mercodia (Uppsala, Sweden) according to the manufacturer's instructions. The absorbance was measured with a microplate reader and the concentration was calculated by comparison with a calibration curve.

2.5. Statistical analysis

All data are represented as means ± standard error and were analyzed with Statcel 3 (OMS, Saitama, Japan). A value of $p < 0.05$ was considered statistically significant. The difference between groups was evaluated by using a one-way ANOVA. If ANOVA indicated a significance difference, a multiple post hoc test was performed to determine the significance between the means.

3. Results

3.1. Body weight and fat weight

Body weights and adipose tissue weights were measured, since insulin resistance basically develops with accumulation of body fat. Although it has been known that the body weight markedly increases with age in OLETF rats compare with normal rats [14], intake of propolis did not affect body weight at 18 week of age (Table 1). Consisting with this phenomenon, the weight of epididymal fat was not significantly changed among the groups.

3.2. Blood glucose, plasma FFA, and plasma ketone body

In OLETF rats, blood glucose levels have been reported to gradually increase after 10 week of age [15]. Propolis significantly decreased the concentration of casual blood glucose in both 0.1%- and 0.5%-propolis ingestion groups compared with normal diet group ($p < 0.01$) (Table 2). On the other hand, concentrations of FFA and β-hydroxy butyrate in plasma were not changed among three groups.

3.3. Blood pressure

Blood pressures are gradually elevated along with increases of blood glucose levels after 10 weeks of age in OLETF rats [15]. Propolis significantly reduced systolic arterial pressures in both 0.1%- and 0.5%-propolis contained diet groups compared with normal diet ($p < 0.05$) (Fig. 1).

3.4. Plasma insulin and aldosterone

Similar to the result of blood glucose levels, the concentration of plasma insulin was also significantly decreased in the 0.5%-propolis diet group compared with normal diet group ($p < 0.05$) (Table 3). The concentration of plasma aldosterone, a hormone affecting blood pressure via regulation of body fluid volume, was not significantly changed by ingestion of propolis.

3.5. pH in interstitial fluid and urine

We next examined the effect of propolis on pH in interstitial fluid and urine because metabolic acidosis occurs with develop-

Table 1
Body weight and adipose tissue weight.

	Normal	0.1%-Propolis	0.5%-Propolis
Body weight (g)	471.3 ± 11.8	505.5 ± 10.9	492.1 ± 8.4
Epididymal fat (g)	4.23 ± 0.47	4.66 ± 0.27	4.12 ± 0.21

Values are the mean ± standard error obtained from six rats. Normal, normal diet group; 0.1%-propolis, 0.1%-propolis diet group; 0.5%,-propolis, 0.5%-propolis diet group.

Table 2
Concentrations of metabolic parameters in plasma.

	Normal	0.1%-Propolis	0.5%-Propolis
Blood glucose (mg/dL)	130 ± 2	115 ± 3**	113 ± 2**
Free fatty acid (mEq/L)	0.74 ± 0.07	0.72 ± 0.04	0.68 ± 0.04
β-Hydroxy butyrate (mEq/L)	1353 ± 99	1622 ± 132	1560 ± 116

Values are the mean ± standard error obtained from six rats. Normal, normal diet group; 0.1%-propolis, 0.1%-propolis diet group; 0.5%-propolis, 0.5%-propolis diet group.
** Significant difference from the normal at the level of $p < 0.01$.

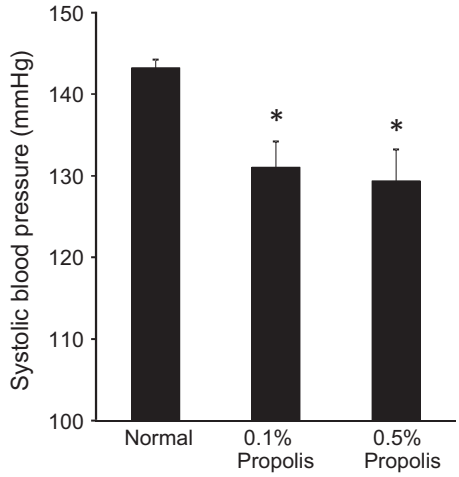

Fig. 1. The effect of propolis on systolic blood pressure in OLETF rats. Values are the mean ± standard error obtained from six rats. Normal, normal diet group; 0.1%-propolis, 0.1%-propolis diet group; 0.5%-propolis, 0.5%-propolis diet group. *Significant difference from the normal at the level of $p < 0.05$.

Table 3
Concentrations of plasma insulin and aldosterone hormone.

	Normal	0.1%-Propolis	0.5%-Propolis
Insulin (μU/L)	6.9 ± 0.5	6.3 ± 0.2	5.4 ± 0.4*
Aldosterone (pg/mL)	225 ± 32	273 ± 40	309 ± 33

Values are the mean ± standard error obtained from six rats. Normal, normal diet group; 0.1%-propolis, 0.1%-propolis diet group; 0.5%-propolis, 0.5%-propolis diet group.
* Significant difference from the normal at the level of $p < 0.05$.

Table 4
Level of pH in interstitial fluid and urine.

	Normal	0.1%-Propolis	0.5%-Propolis
Ascites	7.23 ± 0.02	7.29 ± 0.02	7.34 ± 0.01**
Liver	7.25 ± 0.03	7.40 ± 0.03*	7.35 ± 0.03*
Skeletal muscle	7.36 ± 0.02	7.41 ± 0.03	7.43 ± 0.03
Urine	6.3 ± 0.2	6.4 ± 0.2	6.8 ± 0.2

Values are the mean ± standard error obtained from six rats. Normal, normal diet group; 0.1%-propolis, 0.1%-propolis diet group; 0.5%-propolis, 0.5%-propolis diet group.
* Significant difference from the normal at the level of $p < 0.05$.
** Significant difference from the normal at the level of $p < 0.01$.

ment of insulin resistance. pH in ascites was significantly increased in the 0.5%-propolis diet group compared with normal diet group ($p < 0.01$) (Table 4). We also found a significant increase in interstitial fluid pH of liver tissue fluid in 0.1%- and 0.5%- propolis diet group compared with normal diet group ($p < 0.05$). In addition, pH showed a tendency higher in both interstitial fluid of skeletal muscle and urine by intake of propolis.

4. Discussion

The present study firstly revealed the following observations: (1) propolis decreased levels of blood glucose and plasma insulin, (2) propolis decreased the systolic blood pressure, and (3) propolis potentiated the increase of pH of interstitial fluid, ascites, in OLETF rats. OLETF rats have been known as a model of spontaneous non-insulin-dependent diabetes mellitus. The rats are characterized by hyperphagia, obesity, decreased glucose infusion rate in an euglycemic clamp at 16 weeks of age, and hyperinsulinemia at 24 weeks of age in response to an intravenous glucose infusion and later development of type 2 diabetes [15,16]. In addition, the systolic blood pressure is gradually elevated from under 120 mmHg to over 150 mmHg with age [15]. Thus, our observations suggest that propolis suppressed development of insulin resistance and hypertension. Although previous studies have shown that extracts of propolis attenuate diabetic nephropathy and β-cells damage in model animal experiments [17,18], we here reports a beneficial, preventing effect of propolis on diabetes mellitus in the early stage of developing insulin resistance.

Generally, it is considered that metabolic syndrome is primarily developed on the basis of insulin resistance with accumulated visceral adipose tissue. Adipose tissue secretes bioactive factors, adipocytokines, such as tumor necrosis factor alpha, plasminogen activator inhibitor, and resistin, into circulation [19–21]. Growing evidences have shown that these adipocytokines closely correlate with health problems, such as obesity and metabolic- and cardiovascular-disorders, as these factors cause insulin resistance, injury to the endothelium, and inflammation [22,23]. OLETF rats are characterized by accumulation of body fat compared with normal control rats, which is generally recognized as a major cause in development of insulin resistance. However, in the present study, intake of propolis reduced levels of blood glucose and plasma insulin without a decrease in adipose tissue weight, suggesting that the metabolic improvement of propolis would be not mediated by reduction of visceral fat.

Body fluids of diabetes patients would be acidic mainly due to elevation of ketone body production. In addition, an elevation of lactic acid production in metabolic tissues would be also likely involved in the body fluid acidosis. The organic acids-induced acidosis could contribute to the development of insulin resistance. Several studies [24–26] have suggested a close correlation of organic acid production with insulin sensitivity in both type 2 diabetes patients and healthy subjects. Lower levels of serum bicarbonate and higher levels of anion gap resulted from metabolic acidosis were associated with lower insulin sensitivity [27]. Although the underlying mechanism remains unclear, enzymatic inhibition in glycolytic and oxidative phosphorylation pathways due to decreased pH may be involved in impaired insulin-sensitive glucose uptake. In the present study, we found that intake of propolis increased pH of ascites and metabolic tissues compared with normal diet, suggesting that dietary propolis suppressed production of organic acids or elevated buffering capacity in those tissues. Therefore, propolis may improve insulin sensitivity via preventing metabolic acidosis.

The blood pressure is regulated by various factors in the cardiovascular system. In addition to the cardiovascular system, the regulation of body fluid volume via the Na^+ reabsorption in the distal segment of the renal tubules plays a critical role in the control of blood pressure. The Na^+ reabsorption in the distal segment of the renal tubules is essentially regulated by the renin–angiotensin-aldosterone system via aldosterone-induced stimulation of production and apical membrane expression of epithelial Na^+ channel (ENaC) in epithelial cells of distal renal tubules [28,29]. However, propolis did not change plasma aldosterone level as shown in the present study. On the other hand, previous studies demonstrated that insulin is another regulator of ENaC and can facilitate ENaC-mediated Na^+ transport from apical side to basolateral side in renal epithelial cells by stimulation of ENaC translocation to the apical membrane and/or activation of ENaC [30–33]. Therefore, the reduction of circulating insulin by propolis would suppress the ENaC-mediated Na^+ reabsorption in renal tubules leading to the decreased blood pressure via the control of extracellular fluid volume.

Some components contained in propolis including phenolic acids, flavonoids and amino acids are suggested to be benefit for

prevention and treatment of insulin resistance [33–36]. Although it is difficult to specify which components exert the effects on reduction of levels of blood glucose and plasma insulin shown in the present study, the beneficial effects are more likely when nutrients are taken in combination, rather than a particular nutrient alone. For example, although supplementation with vitamin C or vitamin E alone is difficult to improve insulin resistance in human studies [37,38], their combined intake of the compounds could improve insulin sensitivity [39,40]. Therefore, the results on propolis action observed in the present study might be attributed to integral actions of nutrients but not a single one contained in propolis. However, it is difficult to speculate regarding detail mechanisms responsible for the observed improvement in levels of blood glucose and blood pressure in this study. A comprehensive investigation is necessary to determine the mechanisms behind the beneficial effects of propolis.

In conclusion, we found that propolis improved levels of blood glucose and plasma insulin associated with improvement of body fluid acidosis in the early developmental stage of insulin resistance in OLETF rats. In addition, blood pressures were reduced by intake of propolis, which may be caused by a decrease of ENaC-mediated Na^+ reabsorption in the renal tubules via the reduced insulin level. These observations suggest that propolis extracts prevent insulin resistance and the related metabolic and cardiovascular disorders.

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