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Brazilian propolis extract increases leptin expression in mouse adipocytes

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ABSTRACT

We investigated the anti-obesity effects of Brazilian green propolis ethanol extract using a mouse model of obesity. Repeated intraperitoneal injection of propolis (100 mg/kg twice a week) caused feeding suppression in C57BL/6 mice, whereas this treatment had negligible effects on C57BL/6 *ob/ob* mice. Since C57BL/6 *ob/ob* mice have a missense mutation in the *Lep* gene, leptin is likely to contribute to the propolis-induced feeding suppression. We found that propolis treatment indeed clearly increased leptin mRNA production in the visceral adipose tissues. Moreover, propolis extract directly elevated leptin expression in differentiated 3T3-L1 adipocytes. Artepillin C, an important organic compound found in Brazilian green propolis, failed to induce leptin mRNA in 3T3-L1 cells. Compounds other than artepillin C in Brazilian propolis must thus cause leptin induction in adipocytes, possibly resulting in the suppression of feeding and obesity.

Adiposity and the associated type 2 diabetes are pandemic human health issues. Exploration of procedures for preventing weight gain is thus vitally important for improving quality of life. An adipocytokine leptin, which is released from mature adipocytes, strongly attenuates feeding activity and promotes sympathetic activity (5, 7). Appropriate use of leptin is thus proposed as a treatment for improving metabolic states in obese individuals (5).

Propolis is a hive product of the honeybee (*Apis mellifera*) made up of resinous materials and bee's secretions. Its chemical composition is highly dependent on the location of origin. Brazilian green propolis is mainly made from *Baccharis dracunculifolia* DC (Asteraceae) extract, which contains many biologically active organic compounds in abundance, such as artepillin C (APC). This propolis is recognized as highly effective in the treatment of various conditions, and evidence is accumulating of its health benefits, such as its anti-inflammatory and tumoricidal properties (2, 4, 12).

Brazilian propolis ethanol extract (100 mg/kg i.p., twice per week) caused remarkable feeding repression in C57BL/6 mice (DIO mice; SLC, Hamamatsu, Japan) with diet-induced obesity (60 kcal% high fat diet; Research Diets, New Brunswick, NJ, USA) (Fig. 1A), resulting in attenuation of weight gain (Figs. 1B and 1C). Intraperitoneal injection of propolis thus has an anorexic effect, which might result in an anti-diabetic effect on DIO mice. C57BL/6 *ob/ob* mice (*ob/ob* mice; SLC) exhibited no significant changes in either weight gain or food intake during propolis treatment (Fig. 1C) (10). Since *ob/ob* mice have a missense mutation in the *Lep* gene, this difference between the two strains is likely to be the result of the presence or absence of leptin.

To assess whether propolis extract modulates leptin expression in the adipose tissue, we performed quantitative reverse transcription-PCR (qRT-PCR) analysis, as described by Shimamoto *et al.* (14), of leptin in the epididymal adipose tissue after propolis injection for one month. Propolis extract significantly increased leptin mRNA in the adipose tissues (~2-fold, $P < 0.05$) relative to the vehicle (Fig. 1D).

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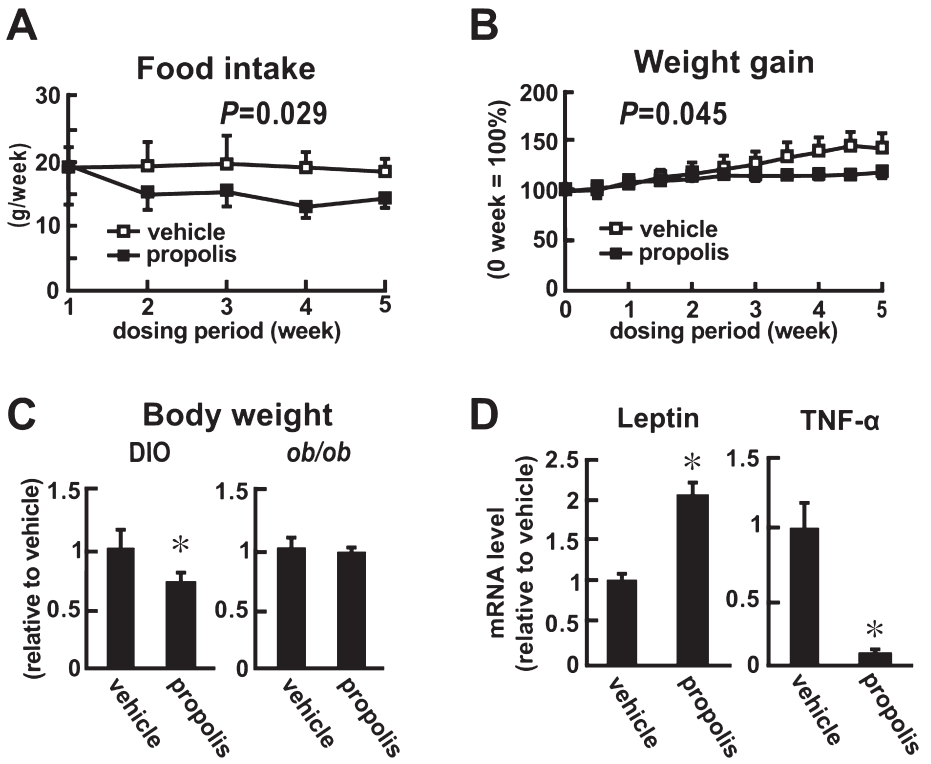

Fig. 1 Effects of intraperitoneal injection of propolis on feeding and leptin expression in adipose tissue. (**A**) Food intake and (**B**) weight gain during treatment with propolis ethanol extract (100 mg/kg i.p.) or vehicle in C57BL/6 mice fed a 60 kcal% high fat diet (DIO mice). Values are mean ± SD of four mice. (**C**) Comparisons of body weight of DIO mice and *ob/ob* mice five weeks after commencing propolis or vehicle injections. Values are mean ± SD of four mice. (A), (B), and (C) reproduced from Kitamura *et al.* 2013 (10). (**D**) Expression of leptin and TNF-*α* in the epididymal adipose tissue of C57BL/6 DIO mice treated with propolis or vehicle. Values are mean ± SD of four mice. $^{*}P < 0.05$.

The propolis extract marginally affected the expression of other adipocytokines, such as adiponectin, apelin, and chemerin (data not shown), but clearly down-regulated TNF-α (Fig. 1C). Intraperitoneal propolis extract thus caused relatively selective induction of leptin in the adipose tissues.

Since leptin is predominantly expressed in adipocytes, propolis might directly induce leptin in adipocytes in mice. To check this, we assessed leptin expression in cultured adipocytes following administration of propolis. Mouse 3T3-L1 cells (Takara, Otsu, Japan) were differentiated using the differentiation cocktail (Takara) containing insulin (10 μg/mL), dexamethasone (2.5 μM), and 3-isobutyl-1-methylxanthine (500 μM) for seven days. Propolis extract (100 μg/mL) was then added to the medium, and after 4 h the cells were subjected to qRT-PCR analysis. Treatment with propolis significantly increased leptin mRNA in 3T3-L1 cells (Fig. 2A). Therefore, propolis is able to directly induce leptin in adipocytes.

A major botanic source of Brazilian green propolis is *Baccharis dracunculifolia* DC, which is rich in APC. In fact, more than 12% of Brazilian propolis is APC. Since APC is reported to have several biological properties, we examined whether APC is responsible for propolis-induced leptin expression in adipocytes. We added 20 μM APC (Wako, Osaka, Japan) to differentiated 3T3-L1 cells, which is comparable to adding 100 μg/mL of the propolis extract. However, leptin mRNA production decreased by about 20% following the addition of APC ($P = 0.018$; Fig. 2B). APC is thus unlikely to be involved in inducing leptin in adipocytes.

In this study, we have shown for the first time that Brazilian propolis ethanol extract has the potential to repress feeding, possibly mediated via the production of leptin in adipocytes. We demonstrated previously that the propolis extract has anti-diabetic effects on *ob/ob* mice (10). In these mice the propolis extract had negligible effects on feeding, indicating that the major targets of the extract are in the periphery of the body. In addition, the extract repressed mild inflammation in the mesenteric adipose tissue. Therefore, Brazilian propolis has potential

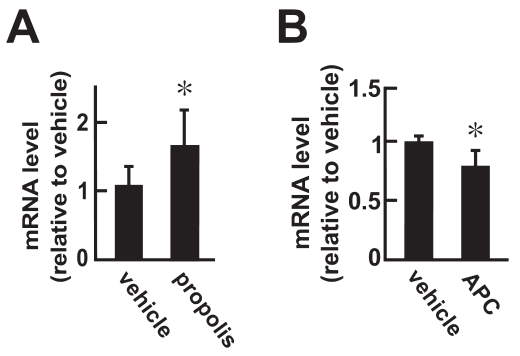

Fig. 2 Effects of propolis extract and APC on leptin expression in differentiated 3T3-L1 cells. Cells were treated with propolis ethanol extract (100 μg/mL) or APC (20 μM) for 4 h and then subjected to qRT-PCR analysis. Values were normalized based on their hypoxanthine phosphoribosyltransferase-1 (HPRT-1) level. Values shown are mean ± SD of four to five dishes of cells. $^*P < 0.05$.

beneficial effects on diabetes through both central (feeding repression via increased production of leptin) and peripheral (anti-inflammation in adipose tissues) mechanisms.

Leptin is an important anti-diabetic adipocytokine that regulates the neural activity of the feeding center in the hypothalamus as well as insulin sensitivity in energy-expenditure tissues. Leptin-deficient mice and mice deficient in leptin receptors thus represent the conditions of spontaneous adiposity and adiposity-associated type 2 diabetes (6). However, it is still arguable whether increased leptin production in adipose tissues is beneficial for adipose tissue inflammation. Leptin causes M2 macrophages to produce M1 macrophage cytokines (1), which potentiates adipose tissue inflammation (4). On the other hand, leptin also accelerates catecholamine signaling, which represses the adipose tissue inflammation elicited by macrophages (11). In this study, propolis administration prevented adiposity and simultaneously induced a significant increase in leptin expression in the adipose tissue of DIO mice. Increased leptin production thus seems to have beneficial effects in this setting.

APC is an important constituent of Brazilian green propolis. This organic compound has a wide variety of biological effects, including immune system modulation and anti-apoptotic effects on neural cells (9, 13). In addition, APC is also postulated to be an anti-diabetic agent. For example, it is a potential ligand for PPARγ, which promotes adipose tissue differentiation and glucose uptake (3). It also inhibits TNF-α-mediated downregulation of adiponectin in differentiated adipocytes, thereby potentially participating in restoring insulin sensitivity (8). In this study, however, APC decreased leptin expression in differentiated 3T3-L1 adipocytes. Further studies are needed to identify the organic compounds in Brazilian green propolis ethanol extract that are responsible for induction of leptin in adipocytes.

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