Eucalyptus leaf extract inhibits intestinal fructose absorption, and suppresses adiposity due to dietary sucrose in rats

Keiichiro Sugimoto¹,², Junko Suzuki³, Kazuya Nakagawa², Shuichi Hayashi², Toshiki Enomoto⁴, Tomoyuki Fujita³, Ryoichi Yamaji¹,³, Hiroshi Inui¹,³⁎ and Yoshihisa Nakano¹,³

¹Center for Research and Development of Bioresources, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan
²Research and Development Center, Nagaoka Perfumery Co. Ltd, Ibaraki, Osaka 567-005, Japan
³Department of Applied Biological Chemistry, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan
⁴Department of Food Science, Ishikawa Agricultural College, Nonoichi, Ishikawa 921-8836, Japan

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Sucrose is more lipogenic than starch, and the extreme ingestion of sucrose induces adiposity and obesity. The aim of this study was to examine the effect of the eucalyptus (Eucalyptus globulus) leaf extract (ELE) on adiposity due to dietary sucrose in rats. In addition, in this study, the effect of ELE on intestinal fructose absorption was also examined. Rats were fed a high-sucrose diet (75 % in calorie base) with or without ELE (10 g/kg diet) for 5 weeks. Body weight was lower in the rats receiving ELE than in the controls (342 (SD 37·9) g (n 7); P<0·05). Furthermore, ELE resulted in decreases in the triacylglycerol concentrations in the plasma (1·44 (SD 0·448) v. 2·79 (SD 0·677) mmol/l (n 7); P<0·05) and liver (19·1 (SD 5·07) v. 44·1 (SD 16·28) μmol/g (n 7); P<0·05). In contrast, ELE did not show any significant effects in the rats fed a starch diet. When rats were orally given ELE 10 min before fructose administration, the intestinal fructose absorption, which was examined by measuring the elevated concentration of fructose in the portal vein at 30 min after the fructose administration, was significantly inhibited in a dose-dependent manner. Furthermore, in rats fed a high-fructose diet, the plasma and hepatic triacylglycerol concentrations were significantly decreased by ELE. These results indicate that ELE, which inhibits the intestinal fructose absorption, can suppress adiposity in rats that ingest large amounts of sucrose or fructose.

Adiposity: Dietary sucrose: Intestinal fructose absorption: Eucalyptus: Eucalyptus globulus

Although the major part of dietary carbohydrate is starch, a large amount of sucrose is used in many foods as a sweetener or other ingredients. In addition, high-fructose corn syrups, which are commercially produced by the enzymatic isomerization of glucose, are utilized as a sweetener in soft drinks and other beverages. After the introduction of high-fructose corn syrups, the consumption of fructose has exponentially increased.

Fructose is more lipogenic than glucose, and the plasma and hepatic triacylglycerol (TG) concentrations are significantly elevated by the extreme ingestion of sucrose or fructose (Fukada et al. 1983). The increased consumption of a fructose-containing diet might be one of the environmental factors contributing to the development of obesity and the accompanying abnormalities of insulin resistance syndrome (Elliott et al. 2002). In addition, dietary fructose results in the elevation of blood pressure, uric acid and lactic acid (Hallfrish, 1990).

Fructose is transported across the intestinal brush-border membranes by the specific transporter, GLUT5, in contrast to glucose, which is absorbed from the lumen of the small intestine by the Na+/glucose co-transporter 1 (SGLT1) (Gould & Holman, 1993; Mahraoui et al. 1994). Thus, if it is possible to inhibit the intestinal fructose absorption specifically, adiposity would be prevented even in subjects who consume not only large amounts of sucrose but also high-fructose corn syrups as a sweetener. However, such inhibitors specific for the intestinal fructose absorption have not yet been found.

Eucalyptus (Eucalyptus globulus) contains a variety of phloroglucinols and hydrolysable tannins. Some of these compounds possess interesting biological activities, such as antioxidation (Amakura et al. 2002), antibacterial (Hou et al. 2000) and antiviral (Takasaki et al. 1990) effects. In Japan, the ‘eucalyptus leaf extract’ is included as one of the natural food additives used for antioxidation in the List of Existing Food Additives (Japanese Ministry of Health and Welfare, 1999). In addition, this plant, although indigenous to Tasmania, is traditionally used to treat diabetes mellitus in South America and Africa.

Abbreviations:
ELE, eucalyptus leaf extract; G6DH, glucose 6-phosphate dehydrogenase; KHK, ketohexokinase; TG, triacylglycerol(s).
⁎ Corresponding author: Dr Hiroshi Inui, fax +81 72 254 9937, email inui@biochem.osakafu-u.ac.jp

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and its antihyperglycaemic effect has been confirmed in streptozotocin-induced diabetic mice (Gray & Flatt, 1998).

The objective of the present study was to examine the effect of the eucalyptus leaf extract (ELE) on adiposity due to dietary sucrose in rats. In addition, in this study, the effect of ELE on intestinal fructose absorption was also examined.

Materials and methods

Preparation of eucalyptus leaf extract

One kilogram of dried eucalyptus (E. globulus) leaves, which were harvested in Spain and purchased from K. Kobayashi & Co. (Kobe, Japan), were extracted with 10kg boiling ethanol–water (1:2, v/v) under reflux for 2h. The obtained extract was filtered, evaporated to dryness in vacuo, and used as ELE throughout the experiments. About 200 g dried ELE was obtained from 1 kg dried leaves.

Diets and animal housing

Male Wistar rats were purchased from Kiwa Laboratory Animals (Wakayama, Japan), and maintained on a starch diet for at least 1 week prior to the study. The starch diet contained the following (1 kg): 170 casein, 700 starch, 30 soya bean oil, 35 mineral mixture (AIN-93G-MX; Clea, Tokyo, Japan), 10 vitamin mixture (AIN-93VX; Clea), 3 methionine, 2 choline chloride and 50 cellulose. In addition to the starch diet, a sucrose or fructose diet, in which the corresponding sugar was included instead of starch, was used as the experimental diet. When ELE was supplemented into these experimental diets, a portion of the cellulose was substituted by ELE. These rats were housed individually at controlled temperature (23°C ± 2°C, humidity (60±10%) and lighting (9:00–21:00 hours), and had free access to the diet and water throughout the experiment. All experimental procedures involving the laboratory animals were approved by the Animal Care and Use Committee of Osaka Prefecture University.

Design of experiments with sucrose-fed rats (Expts 1 and 2)

In Expt 1, the effect of ELE on the adiposity was studied in rats fed sucrose as a major energy source. Rats (5 weeks old; 142 (SD 8) g (n 28)), which had been maintained on the starch diet for 1 week, were randomly divided into four groups (seven rats in each group), and fed the starch or sucrose diet with or without ELE (10 g/kg diet) for 5 weeks. During the feeding, the body weight change and food intake were determined. At the end of the feeding, these rats were starved for 16 h, and then anaesthetized with diethyl ether. Blood was sampled from the tail vein, and the concentrations of glucose and TG in the plasma were determined. In addition, the liver and epidydimal adipose tissue were obtained and weighed, and TG concentration in the liver was measured.

In Expt 2, for further elucidation of the preventive effect of ELE on the adiposity induced by dietary sucrose, rats (5 weeks old; 152 (SD 8) g (n 25)), divided into five groups (each five rats), were fed the sucrose diet containing varied amounts of ELE (0, 0.3, 1, 3 or 10 g/kg diet). After feeding for 2 weeks, these rats were starved for 16 h, and liver was excised under diethyl ether anaesthesia to determine the TG concentration and ketohexokinase (KHK) and glucose 6-phosphate dehydrogenase (G6PDH) activities.

Determination of glucose and triacylglycerols in plasma and liver

The plasma glucose concentration was measured by a glucose oxidase method using a commercially available kit (Glucose B-Test; Wako, Osaka, Japan). TG in the plasma and liver were extracted with isopropanol and determined by an acetylacetone method using a commercial kit (Triglyceride-Test; Wako) according to the manufacturer’s instructions.

Enzyme assays

The liver was homogenized with a polytron homogenizer in 25 mmol/l HEPES–KOH buffer, pH 7.4, containing 0.15 mol/l KCl at 4°C. After centrifugation at 10 000 g for 20 min, the obtained supernatant was used as a crude enzyme solution for the assays of KHK and G6PDH. The KHK (Bais et al. 1985) and G6PDH (Glock & MaLean, 1953) activities were measured as references. The protein concentration was determined according to Bradford (1976) using bovine serum albumin as the standard.

Measurement of the sucrase inhibitory activity of eucalyptus leaf extract in vitro (Expt 3)

A crude sucrase solution was prepared from the rat small intestine as previously described (Takenoshita et al. 1998). The sucrase reaction was carried out at 37°C for 30 min using a reaction mixture (1 ml) containing 50 mmol/l potassium phosphate buffer, pH 6.5, 25 mmol/l sucrose and the crude enzyme solution. To examine the sucrase inhibitory activity of ELE, ELE was included at 1, 3 or 10 mg/ml in the reaction mixture. (+)-Catechin (1, 3 or 10 mg/ml) or (−)-epigallocatechin 3-O-gallate (0.05, 0.1 or 0.2 mg/ml) was also added to the reaction mixture as a sucrase inhibitor. The sucrase reaction was stopped by heating in a boiling water bath for 2 min, and the amount of glucose formed during the reaction was measured by a hexokinase–G6PDH method using a commercial kit (Roche Diagnostics).

Determination of the inhibitory effect of eucalyptus leaf extract on intestinal fructose absorption (Expt 4)

Rats (8 weeks old; 307 (SD 15) g (n 25)), which had been maintained on the starch diet, were starved for 16 h, and orally given ELE (0, 0.1, 0.3 or 1 g/kg body weight; dissolved in 0.5 ml water). Ten minutes later, these rats were orally administered the solution with or without fructose (2 g/kg body weight; dissolved in 0.5 ml water). Blood (about 50 μl) was sampled from the portal vein under diethyl ether anaesthesia at 30 min after the administration of fructose, and the fructose concentration in the plasma was determined by the fructose dehydrogenase method described by Ameyama et al. (1981) with some modifications. Briefly, plasma sample (10 μl) was incubated in a reaction mixture (1 ml) containing 0.1 mol/l potassium phosphate buffer, pH 6.0, 1% (v/v) Triton X-100, 0.1 mmol/l WST-1 (Dojindo, Kumamoto, Japan), 8 μmol/l 1-methyl phenazine methosulphate (Dojindo), 5 units/ml fructose dehydrogenase (from Gluconobacter sp.; Toyobo, Osaka, Japan), at 30°C for 3 h. The concentration of fructose was estimated by measuring the increase in the absorbance at 438 nm due to the reduction of WST-1.
Suppression of adiposity by eucalyptus

Effects of eucalyptus leaf extract on sucrose-fed rats (Expts 1 and 2)

Rats were fed a diet containing starch or sucrose as the major energy source (about 75% total energy) for 5 weeks, and the effect of ELE, which was supplemented into the diet (10 g/kg diet), on the body weight gain was examined (Expt 1). As shown in Fig. 1, no significant difference in the body weight gain was observed between the ELE and control groups on the starch diet. However, for the rats fed the sucrose diet, the body weight increase was significantly suppressed by the addition of ELE into the diet. The dietary sucrose resulted in an enlarged liver, and the liver weight, even normalized to the body weight, was significantly greater in the sucrose-fed rats, compared with the starch-fed ones (Table 1). In addition, the epididymal adipose tissue weight, normalized to the body weight, was also increased in the sucrose-fed rats (P < 0.05). These changes in the liver and epididymal adipose tissue weights due to the dietary sucrose were coupled with the oxidation of fructose during the incubation. To examine the intestinal glucose absorption, rats (8 weeks old; 295 (SD 10.6) g (n 15)) were administered glucose (2 g/kg body weight) instead of fructose, and the glucose concentration in the portal vein was measured using the Glucose B-Test.

Fig 1. Effect of eucalyptus leaf extract (ELE) on body weight increase in rats fed sucrose (Expt 1). Rats (5 weeks old) were fed the starch (C, A) or sucrose (O, ●) diet with (ELE group ●, ●) or without (control group C, A) ELE (10 g/kg diet) for 5 weeks, and body weight change was followed during the feeding. Values are the means of seven rats with their standard deviations. *P < 0.05 v. the control group fed the same diet at the same time point.

Sucrase inhibition by eucalyptus leaf extract (Expt 3)

The inhibitory effect of ELE on the intestinal sucrose activity was examined in vitro, and was compared with those of the well-known sucrase inhibitors, (+)-catechin and (−)-epigallocatechin...
As shown in Table 2, sucrase activity was inhibited by ELE in a concentration-dependent manner, and a 50% inhibition was observed at about 3 mg/ml. However, the inhibitory effect of ELE was weaker when compared with either catechin or epigallocatechin 3-O-gallate.

Inhibitive effect of eucalyptus leaf extract on intestinal fructose absorption (Expt 4)

Rats were orally given ELE 10 min before the administration of fructose, and the effect of ELE on the intestinal fructose absorption, which was determined by measuring the elevated concentration of fructose in the portal vein, was examined. In the rats treated without ELE, the fructose concentration in the portal vein was greatly elevated at 30 min after the fructose administration (Fig. 4). The elevation of the fructose concentration was significantly inhibited by the treatment with ELE in a dose-dependent manner, and a 45% inhibition was observed at a dose of 0.1 g/kg body weight. In contrast, when glucose was substituted for fructose, the elevation of the glucose concentration in the portal vein was barely affected by the treatment with ELE even at the high dose of 1 g/kg body weight.

Fig. 2. Hepatic triacylglycerol (TG) concentration in rats fed the sucrose diet containing varied amounts of eucalyptus leaf extract (ELE) (Expt 2). Rats (5 weeks old) were fed the sucrose diet containing varied amounts of ELE (0–10 g/kg of diet) for 2 weeks, and the hepatic concentration of TG was determined. Values are the means of five rats with their standard deviations shown by vertical bars. Values with unlike letters were significantly different (P<0.05).

Fig. 3. Hepatic ketohexokinase (KHK) and glucose 6-phosphate dehydrogenase (G6PDH) activities in rats fed the sucrose diet containing varied amounts of eucalyptus leaf extract (ELE) (Expt 2). Rats (5 weeks old) were fed the sucrose diet containing varied amounts of ELE (0–10 g/kg diet) for 2 weeks, and the hepatic KHK (A) and G6PDH (B) activities were determined. Values are the means of five rats with their standard deviations shown by vertical bars. Values with unlike letters were significantly different (P<0.05).
Effects of eucalyptus leaf extract on fructose-fed rats (Expt 5)

To explore whether ELE can effectively suppress adiposity induced by dietary fructose, rats were fed a high-fructose diet that contained ELE at 10 g/kg diet for 5 weeks. As shown in Table 3, the body weight of the fructose-fed rats was not significantly changed by the addition of ELE to the diet. In addition, the liver weight, plasma glucose concentration and food intake were barely different between the ELE and control groups. However, the epididymal adipose tissue weight (normalized to the body weight) was significantly lower in the ELE group than in the control. Furthermore, the supplementation of ELE resulted in a significant reduction of the plasma and hepatic concentrations of TG.

Discussion

Fructose absorbed in the small intestine is delivered mainly to the liver. The first step of the fructose metabolism in the liver is its phosphorylation by the action of KHK (fructokinase) to produce fructose 1-phosphate (Mayes, 1993). The produced fructose 1-phosphate is split by aldolase B into glyceraldehyde and dihydroxyacetone phosphate, a member of the intermediates in the glycolytic sequence. The third enzyme of the fructose metabolism is triokinase, which catalyses the phosphorylation of glyceraldehyde by ATP to form glyceraldehyde 3-phosphate, another intermediate of the glycolytic pathway. Thus, one fructose molecule is metabolized into two triose phosphates that bypass the main rate-controlling step in glycolysis, 6-phosphofructokinase.

Fructose can provide carbon atoms for both the glycerol and acyl portions of the TG molecules. High concentrations of fructose can serve as a relatively unregulated source of acetyl-CoA, in contrast to glucose whose uptake and metabolism are regulated as the level of 6-phosphofructokinase, which is inhibited by citrate and ATP (Mayes, 1993). Therefore, fructose is more lipogenic than glucose, and the plasma and hepatic concentrations of TG are significantly elevated when large amounts of fructose or sucrose are fed to rats (Fukuda et al., 1983).

In this study, the plasma and hepatic concentrations of TG elevated in the rats fed the sucrose diet were significantly decreased when ELE was added to the diet (Table 1; Fig. 2). In contrast, ELE did not show any significant effects on the plasma and hepatic TG concentrations in the starch-fed rats. In addition, the body weight gain and epidydimal adipose tissue weight were also significantly reduced by ELE in the sucrose-fed rats (but not in the starch-fed ones) (Fig. 1 and Table 1). These results indicate that dietary sucrose-induced lipogenesis and obesity are suppressed when ELE is ingested. Food intake was not affected by ELE in the sucrose-fed rats as well as the starch-fed ones (Table 1), indicating that ELE does not have toxic effects on the rats that might inhibit food intake, and therefore, body weight gain.

It has been reported that KHK activity is enhanced in the liver to stimulate fructose metabolism when rats are fed sucrose or fructose (Fukuda & Iritani, 1984; Hallfrish, 1990; Werman & Bhathena, 1995). In addition, the feeding of sucrose or fructose results in increases in the lipogenic enzymes, such as fatty acid synthase, acetyl-CoA carboxylase and G6PDH, in the liver (Fukuda et al., 1983; Iritani, 1992; Osaki et al., 2001). The KHK was inhibited by ELE in a dose-dependent manner, suggesting that ELE may suppress fructose-induced lipogenesis by inhibiting KHK activity in the liver.

Table 2. Inhibition of intestinal sucrase activity by eucalyptus leaf extract (ELE) in vitro (Expt 3)*

<table>
<thead>
<tr>
<th>Relative activity (%)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>5.87</td>
</tr>
<tr>
<td>ELE 1 mg/ml</td>
<td>77.2</td>
<td>2.18</td>
</tr>
<tr>
<td>3 mg/ml</td>
<td>50.3</td>
<td>2.14</td>
</tr>
<tr>
<td>10 mg/ml</td>
<td>21.0</td>
<td>4.26</td>
</tr>
<tr>
<td>Catechin 1 mg/ml</td>
<td>57.1</td>
<td>6.79</td>
</tr>
<tr>
<td>3 mg/ml</td>
<td>33.2</td>
<td>5.74</td>
</tr>
<tr>
<td>10 mg/ml</td>
<td>10.1</td>
<td>0.26</td>
</tr>
<tr>
<td>Epigallocatechin 3-O-gallate 0.05 mg/ml</td>
<td>67.4</td>
<td>3.41</td>
</tr>
<tr>
<td>0.1 mg/ml</td>
<td>52.2</td>
<td>1.76</td>
</tr>
<tr>
<td>0.2 mg/ml</td>
<td>32.8</td>
<td>3.88</td>
</tr>
</tbody>
</table>

*Sucrase activity was determined in vitro in the presence of ELE, (+)-catechin or (+)-epigallocatechin 3-O-gallate at the concentration cited. Data are presented as percentage of control (without any inhibitors).
Table 3. Suppressive effect of eucalyptus leaf extract (ELE) on adiposity in rats fed the fructose diet (Expt 5)†

(Means with their standard deviations for seven rats in each group)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>ELE group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>394</td>
<td>25·9</td>
</tr>
<tr>
<td>Liver weight (% of body weight)</td>
<td>3·47</td>
<td>0·274</td>
</tr>
<tr>
<td>Adipose tissue weight (% of body weight)</td>
<td>1·76</td>
<td>0·298</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>8·87</td>
<td>0·680</td>
</tr>
<tr>
<td>Plasma TG (mmol/l)</td>
<td>2·50</td>
<td>0·488</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>817</td>
<td>31·3</td>
</tr>
</tbody>
</table>

† Rats (5 weeks old; 152 (SD 5·2) g (n 14)) were fed the fructose diet without (control group) or with (ELE group) ELE (10 g/kg diet) for 5 weeks, and the body weight, liver and epidydimal adipose tissue weights (normalized to the body weight), plasma glucose concentration, plasma and hepatic TG concentrations, and food intake were determined.

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**References**


