

Eucalyptus Leaf Extract Suppresses the Postprandial Elevation of Portal, Cardiac and Peripheral Fructose Concentrations after Sucrose Ingestion in Rats

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Received 29 September, 2009; Accepted 5 December, 2009; Published online 20 March, 2010

Summary Overintake of sucrose or fructose induces adiposity. Fructose undergoes a strong Maillard reaction, which worsens diabetic complications. To determine whether *Eucalyptus globulus* leaf extract (ELE) suppresses the postprandial elevation of serum fructose concentrations (SFCs) in the portal, cardiac, and peripheral blood after sucrose ingestion, we performed gas chromatography/mass spectrometry (GC/MS) and measured SFC without any interference by contaminating glucose in the samples. Fasting Wistar rats were orally administered water (control group) or ELE (ELE group) before sucrose ingestion. Blood was collected from the portal vein, heart, and tail. The increase in the SFCs in the portal and cardiac samples 30 min after sucrose ingestion was lower in the ELE group than in the control group. The coefficient of correlation between the SFCs in the portal and cardiac samples was 0.825. The peripheral SFC in the control group progressively increased and was 146 $\mu\text{mol/L}$ at 60 min. This increase was significantly lower in the ELE group. In contrast, the serum glucose concentrations in the 2 groups were similar. ELE suppressed postprandial hyperfructosemia in the portal, cardiac, and peripheral circulations. ELE may counteract glycation caused by high blood fructose concentrations induced by the consumption of fructose-containing foods or drinks.

Key Words: *Eucalyptus globulus*, serum fructose concentration, postprandial hyperfructosemia, intestinal fructose absorption, sucrose-tolerance test

Introduction

While starch is the major component of dietary carbohydrate, sucrose is also ingested in large amounts because it is used in many foods as a sweetener or for other purposes.

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In addition, the consumption of free fructose has exponentially increased since high-fructose corn syrups (HFCSs) began to be used as sweeteners in soft drinks and processed foods [1, 2]. In the United States of America, added sugars account for more than 30% of the calorie intake of heavy consumers of soft drinks, and it is presumed that these consumers ingest approximately 100 g/day of fructose [3].

Fructose is more lipogenic than glucose, and serum and hepatic triacylglycerol concentrations are significantly elevated after excessive consumption of sucrose or fructose [4]. The increased consumption of dietary fructose may be one of the factors that contributes to the development of obesity and the abnormalities that accompany the insulin-resistance syndrome [5–7].

Emerging evidence suggests that excessive consumption of dietary fructose is an important factor contributing to the increased prevalence of metabolic syndrome among people in the United States. In addition, the incidence of soft-drink ketosis, which is caused by frequent long-term consumption of commercial beverages containing HFCS, has increased among young obese men in Japan [8, 9]. A position statement issued by the American Diabetes Association discourages the use of fructose as a sweetener in the diet of a diabetic patient because fructose adversely affects serum lipid concentrations [10]. The adverse effects of dietary sucrose can be prevented by inhibiting the sucrase reaction in the small intestine [11]. However, sucrase inhibitors are ineffective when HFCSs are consumed as sweeteners.

The evergreen tree *Eucalyptus globulus* is native to Australia and is widely distributed around the world. In South America and Africa, the leaves of this plant, from which tea is made, are used as a traditional remedy for diabetes. The antihyperglycemic effect of *E. globulus* leaf extract (ELE) has been confirmed in mice with streptozotocin-induced diabetes [12]. Furthermore, in a previous study [13], we reported that the aqueous ethanolic extract of eucalyptus leaves reduces intestinal fructose absorption and thereby inhibits the development of fatty liver, the accumulation of visceral fat, and the increase in serum triglyceride concentrations induced by excessive sucrose consumption, although the active ingredients of ELE have not yet been identified. To the best of our knowledge, no agents other than ELE that specifically inhibit intestinal fructose absorption have been identified thus far.

In the abovementioned study, we evaluated changes in the fructose content of the portal blood by using an enzymatic method [13], which is not highly specific for fructose. Further, the effect of ELE on circulating blood has not yet been investigated. Because of these reasons, in this study, we used a highly sensitive method [14] to accurately measure the postprandial fructose concentrations in serum derived from portal and cardiac blood after the oral administration of sucrose with or without ELE in rats (Study 1). The effects

of ELE on changes in the peripheral serum fructose concentration (SFC) were also estimated (Study 2).

Materials and Methods

Preparation of ELE

ELE was prepared according to a previously described method [13]. The composition of ELE used in the study was as follows: total polyphenols measured as tannic acid equivalents (Folin-Ciocalteu's method) [15], 30%; moisture (normal pressure at 105°C and 3 h), 3.0%; ash (produced by direct ashing), 5.7%; protein measured as leucine equivalents (Kjeldahl method), 1.6%; and lipid (Röse-Gottlieb method), 5.7%.

Experimental animals

Male Wistar rats (age, 8 weeks) were purchased from Kiwa Laboratory Animals (Wakayama, Japan). The animals were housed in cages (4–5 per cage) under standard laboratory conditions of a 12/12-h light-dark cycle (light phase beginning at 6.00 AM), an ambient temperature of 23°C ± 2°C, and humidity of 60% ± 10%. All animals received a standard diet (CE-2, Clea Japan Inc., Tokyo, Japan) and water *ad libitum* for at least 1 week prior to the study. All experimental procedures involving the laboratory animals were approved by the Animal Care and Use Committee of Osaka Prefecture University. In this study, we performed the 2 following experiments:

Effect of ELE on changes in postprandial fructose concentrations in serum samples derived from portal and cardiac blood (Study 1)

Rats (body weight, 271 ± 23 g; *n* = 31) were starved for 16 h. Twenty-five rats were orally administered water (control group) or an ELE suspension (ELE group; 1 g/kg body weight, dissolved in 0.5 mL water). After 10 min, these rats were orally administered a sucrose solution (2 g/kg body weight, dissolved in 0.5 mL water). Under diethyl ether anaesthesia, portal and cardiac blood were sampled from the portal vein (approximately 100 µL) and the heart (>1 mL) 30 and 60 min after the sucrose ingestion. Six rats in the fasting state were operated on under diethyl ether anaesthesia, and their portal and cardiac blood were sampled.

Effect of ELE on changes in postprandial fructose concentrations in peripheral serum (Study 2)

An oral sucrose-tolerance test was conducted using the same protocol as in Study 1. In brief, rats (body weight, 274 ± 21 g; *n* = 10) were fasted and orally administered water or the ELE suspension. After 5 min, these rats were orally administered the sucrose solution. Before the administration of the test sample, and 30 and 60 min after sucrose

ingestion, peripheral blood (approximately 0.2 mL) was sampled from the tail, without anaesthesia, by using 26-G needles.

Blood variables

The SFCs in the portal, cardiac, and peripheral blood samples in Studies 1 and 2 were accurately determined using gas chromatography/mass spectrometry (GC/MS), with $^{13}\text{C}_6$ -fructose as an internal standard [14]. The serum glucose concentrations in the cardiac and portal samples ($n = 31$) were measured using the Glucose B test kit (Wako Pure Chemical Industries, Osaka, Japan).

Statistical analysis

Values are presented as mean \pm SD. Statistical analyses were performed using GB-Stat 5.4 (Dynamic Microsystems, Silver Spring, MD). We analyzed the fructose and glucose

concentrations in the serum samples derived from portal and cardiac blood prior to and 30 min after sucrose ingestion by using one-way analysis of variance (ANOVA), followed by the Tukey *post hoc* test. The changes in the peripheral SFCs were compared between the ELE and control groups by using two-way ANOVA for repeated measures and Bonferroni multiple comparison test for *post hoc* analyses.

Results

Effect of ELE on changes in the postprandial SFCs in samples derived from portal and cardiac blood (Study 1)

As shown in Fig. 1, the fasting SFCs in the samples derived from portal (A) and cardiac blood (B) were $114 \pm 77 \mu\text{mol/L}$ and $19.8 \pm 7.0 \mu\text{mol/L}$, respectively. In the control group, after oral sucrose administration, the SFC in the samples derived from portal blood increased transiently,

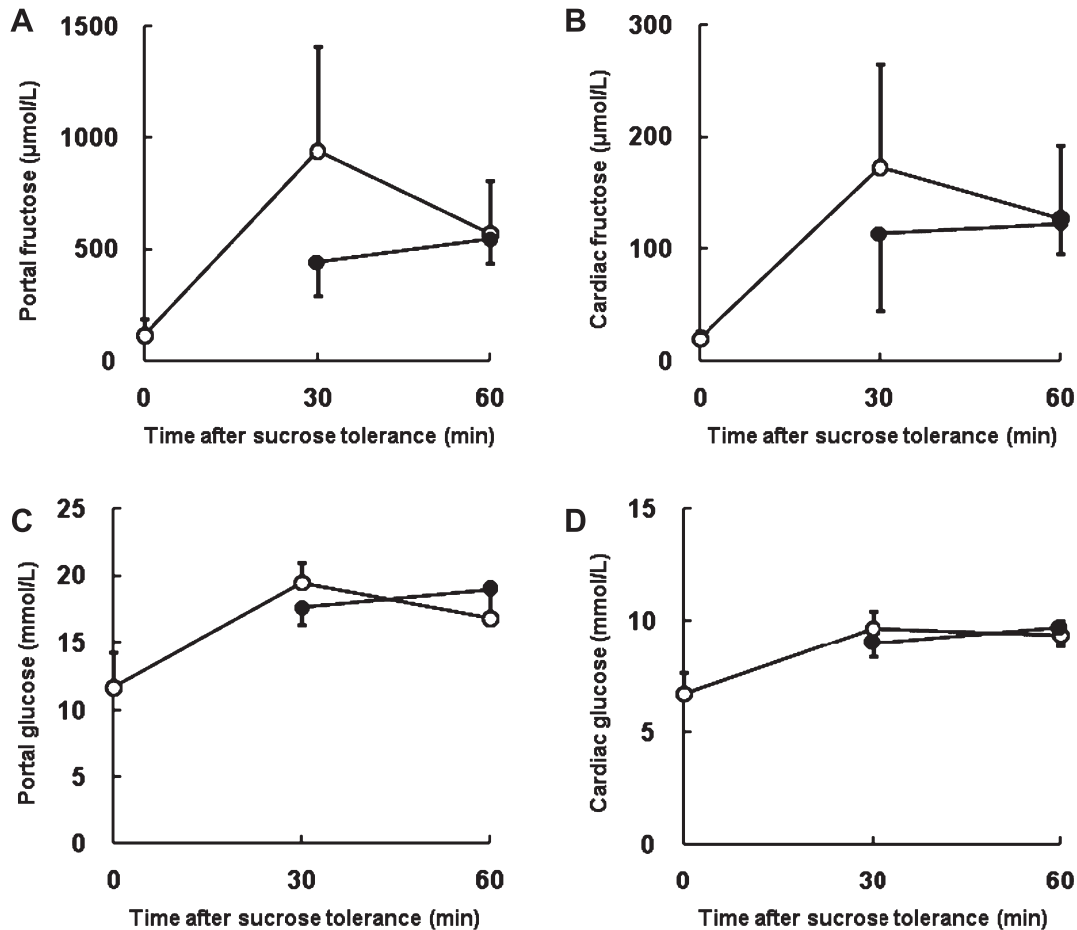


Fig. 1. Eucalyptus leaf extract (ELE) suppresses the postprandial elevation of serum fructose concentrations in samples derived from portal (A) and cardiac (B) blood and does not affect the glucose concentrations (C and D) after a sucrose-tolerance test in rats (Study 1). Mean (SD) fructose and glucose concentrations in the portal and cardiac samples in the control (open circles) and ELE (closed circles) groups. Fasting rats ($n = 31$) were orally administered water (control group) or ELE at a dose of 1 g/kg body weight (ELE group) prior to the ingestion of sucrose (2 g/kg body weight). The fructose and glucose concentrations in the portal and cardiac samples were determined.

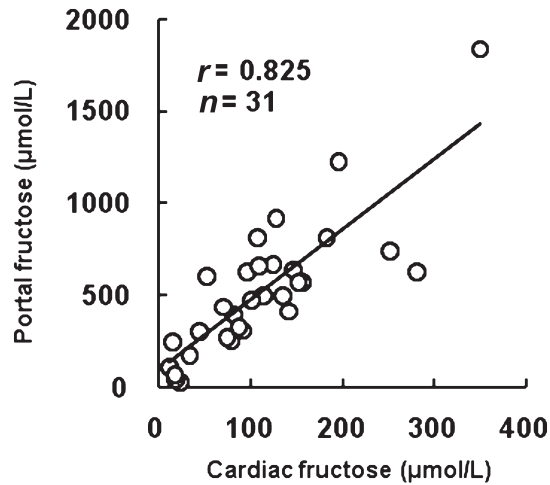


Fig. 2. Correlation between the serum fructose concentrations in the portal and cardiac samples during the sucrose-tolerance test (Study 1). Individual fructose concentrations in the portal and cardiac samples in both the control and eucalyptus leaf extract (ELE) rats (open circles). The coefficient of correlation was 0.825 ($n = 31$).

peaked at 30 min, and then decreased. These changes were consistent with the changes in the SFC in the samples derived from cardiac blood, except that the SFC in the cardiac samples at 30 min was considerably lower than that in the portal samples. In the ELE group, the increase in the SFC in the portal samples was significantly lower than that in the control group ($p < 0.05$), and the inhibition ratio at 30 min was 53%. The SFC in the cardiac samples was also lower than that in case of the control group, but not significantly so. The correlation between the SFCs in the portal and cardiac samples is shown in Fig. 2. The coefficient of correlation between these variables ($n = 31$) was 0.825. The changes in the serum glucose concentrations in both portal and cardiac samples during the sucrose-tolerance test were similar in the 2 groups (Fig. 1C and D).

Effect of ELE on changes in the postprandial fructose concentrations in the serum samples derived from peripheral blood (Study 2)

The time course of the peripheral SFCs during the sucrose-tolerance test is shown in Fig. 3. The fasting SFCs in the 2 groups almost equaled the SFC in the cardiac samples in Study 1 (approximately 20 $\mu\text{mol/L}$). After the sucrose load, the peripheral SFC in the control group progressively increased and reached 146 ± 29 $\mu\text{mol/L}$ at 60 min. Although the peripheral SFCs in the ELE group also increased, the increment was significantly lower, and the inhibition ratios were 90% and 65% at 30 and 60 min, respectively ($p < 0.05$).

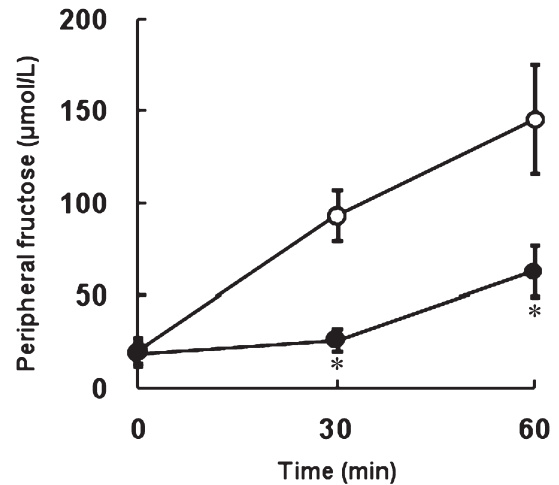


Fig. 3. Eucalyptus leaf extract (ELE) significantly suppresses the postprandial elevation of the peripheral serum fructose concentration after the sucrose-tolerance test in rats (Study 2). The mean (SD) peripheral serum fructose concentrations in the control (open circles) and ELE (closed circles) groups are shown. Fasting rats ($n = 10$) were orally administered water (control group) or ELE at a dose of 1 g/kg body weight (ELE group) prior to the ingestion of sucrose (2 g/kg body weight). The fructose concentrations in the peripheral serum sampled from the tail were determined. * $p < 0.05$ compared to the control group.

Discussion

The results of Studies 1 and 2 show that during the sucrose-tolerance test, ELE suppressed the increase in postprandial SFCs in not only portal samples but also cardiac samples.

Changes in the fructose concentration in the peripheral blood after oral fructose administration have been examined in rats by using an enzymatic method [16] and in human subjects by using a colorimetric method [17–21]. In the case of these methods, other reducing substances in the blood complicate the interpretation of the fructose analysis; for example, high glucose content interferes with the measurement of fructose concentrations. Thus, these substances reduce the sensitivity and accuracy of these methods. In the case of the enzymatic method used by Sugimoto *et al.* [13], the calibration curve was deflected at low fructose concentrations because of coexisting reducing substances (data not shown). It is necessary to measure blood fructose concentrations by using highly sensitive measurement methods because the fructose concentration in peripheral blood is generally low [18, 19]. We developed a highly sensitive method for the measurement of SFCs: complete exclusion of blood glucose by using a normal-phase high-performance liquid chromatography (HPLC) column followed by GC/MS, with $^{13}\text{C}_6$ -

fructose as an internal standard [14]. By using this method, we found that the fasting SFCs in the samples derived from the cardiac (Study 1) and peripheral blood (Study 2) of rats were approximately 20 $\mu\text{mol/L}$ at the baseline and nearly equaled previously reported fasting SFCs in humans [14, 22].

After oral sucrose administration, the SFC in the portal samples in the control group rapidly increased. In our study, ELE suppressed the increase in the SFC after sucrose administration (Fig. 1A). The changes in the SFCs in the cardiac samples (Fig. 1B) were consistent with the changes in the portal samples (Fig. 1A); ELE also suppressed the elevation in the postprandial fructose concentrations in the cardiac samples. Since the SFCs in the portal and cardiac samples were highly correlated (Fig. 2), it was possible to evaluate the suppressive effect of ELE on the increase in postprandial blood fructose concentrations by using cardiac blood, which can be collected in large amounts (>1 mL). The increase in the peripheral SFCs in the ELE group was significantly lower than that in the control group (Fig. 3). Thus, the suppressive effect of ELE can also be evaluated by sampling peripheral blood. In contrast, the changes in the serum glucose concentrations in the cardiac and portal samples did not differ between the ELE and control groups during the 60-min oral sucrose-tolerance test (Fig. 1C and D). A previous study has reported that ELE does not inhibit intestinal glucose absorption in rats, and that it inhibits sucrase activity *in vitro* to a lower extent than other inhibitors such as (+)-catechin and (-)-epigallocatechin 3-*O*-gallate [13]. These results reinforce the hypothesis [13] that ELE specifically inhibits fructose absorption in the intestine. However, the possibility that ELE only delays fructose absorption in the intestine and has little effect on the total amount of absorbed fructose cannot be ruled out because we did not examine the area under the blood concentration-time curve of fructose in this study.

Fructose transported to the liver is rapidly metabolized because the metabolic pathway involved bypasses the main rate-controlling step in glycolysis, which is catalyzed by 6-phosphofructokinase [23]. Consequently, the postprandial fructose concentrations in the peripheral blood are lower than the glucose concentrations [18, 19]. In the control group, the increase in the postprandial SFC in the peripheral samples (Fig. 3) was much lower than that in the portal samples (Fig. 1A). Therefore, we inferred that the liver actively metabolizes fructose. Further, we found that in the control group, the administration of sucrose at a dose of 2 g/kg body weight increased the peripheral SFC to more than 7-fold the fasting concentration. In human subjects, the SFC after the administration of fructose at a dose of 0.75 g/kg body weight has been reported to be approximately 600 $\mu\text{mol/L}$ [21]. In a previous study, we reported the case of a patient who had an SFC of 517 $\mu\text{mol/L}$ because of excessive daily and long-term consumption of soft drinks

[9]. It appears that a large amount of free fructose is released in the systemic circulation by the liver, without being metabolized.

SFCs can significantly affect protein glycation [24] because fructose is more reactive than glucose in the Maillard reaction by at least 1 order of magnitude [25, 26]. Clinical and rodent studies have shown that dietary fructose consumption increases fructated advanced glycation end-products (AGEs) in serum and various tissues [27–29]. ELE may prevent such glycation by lowering postprandial elevation of blood fructose concentration.

The active ingredients of ELE have not yet been identified. Various components have been isolated from ELE such as hydrolyzable tannins [30–32], flavonol glycosides [32, 33], β -diketones [34], and phloroglucinol derivatives [35, 36]. ELE was solubilized in water and subjected to extraction with diethyl ether, ethyl acetate and *n*-butanol; the *n*-butanol extract inhibited intestinal fructose absorption (data not shown). We speculate that the active component(s) of ELE are not β -diketones because these compounds were not identified in our *n*-butanol extract. Further investigation is required to identify the substance(s) in ELE that inhibit intestinal fructose absorption.

In conclusion, ELE can potentially prevent and treat disorders induced by excessive fructose intake. Further, the suppressive effect of ELE on the increase in postprandial blood fructose concentrations may be evaluated in human subjects by using peripheral blood samples.

Acknowledgments

A part of this research was supported by the research and development support program for regional revitalization in the food industry sector from the Ministry of Agriculture, Forestry and Fisheries, Japan. The other expenditure came from Nagaoka Perfumery Co. Ltd.

Abbreviations

HFCS, high-fructose corn syrup; ELE, eucalyptus leaf extract; SFC, serum fructose concentration; GC/MS, gas chromatography/mass spectrometry; HPLC, high-performance liquid chromatography; AGE, advanced glycation end-product.

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