Plasma Insulin Concentration was Increased by Long-Term Ingestion of Guava Juice in Spontaneous Non-Insulin-Dependent Diabetes Mellitus (NIDDM) Rats

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To investigate whether long-term ingestion of guava juice has anti-diabetes and/or anti-obese actions, we employed spontaneous non-insulin-dependent diabetes mellitus Otsuka Long-Evans Tokushima Fatty (OLETF) rats and its control strain Long-Evans Tokushima Otsuka (LETO) rats. Thirty rats of each strain were divided into three groups consisting of glucose, vitamin E, and guava juice ingestion groups. Ingestion of these test solutions was continued from 9 to 32 weeks old. Serum lipid parameters including total cholesterol, triglyceride, free fatty acid, and high-density lipoprotein (HDL)-cholesterol were measured. Oral glucose tolerance test (OGTT) was performed at 32 weeks old rats and at 42 weeks old rats (10 weeks after discontinue of ingestion of guava juice), and then blood glucose levels and plasma insulin concentrations were measured. There were no significant differences in body weight, the amount of food intake and the volume of drink among the groups in OLETF rats. Although the blood glucose level in the guava juice group was not changed as compared with the glucose group, the amount of initial insulin secretion was significantly increased in OLETF rats and was restored by discontinue of ingestion of guava juice. Therefore, the long-term ingestion of guava juice may increase plasma insulin concentration in OLETF rats.

Key words —— guava juice, type II diabetes mellitus, Otsuka Long-Evans Tokushima Fatty rats, blood glucose, insulin

INTRODUCTION

A guava (*Psidium guajava L.*) leaf is drunk as guava tea for the purpose of medical treatment of diabetes mellitus among the people for many years. Guava leaf extract contains guava polyphenol[1] that has an anti-oxidization action.[2] It is known that much guava polyphenol is contained in guava fruit juice rather than a guava leaf extract. In this research, we investigated whether long-term ingestion of guava fruit juice has anti-diabetes and/or the anti-obese effects using spontaneous non-insulin-dependent diabetes mellitus (NIDDM) and mild obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats.[3]

MATERIALS AND METHODS

Study Design —— All animal studies were reviewed and approved by the Animal Care Committee at the University of the Ryukyus. OLETF (diabetic rats) and non-diabetic Long-Evans Tokushima Otsuka (LETO, normal rats) at 6 weeks old were obtained from the Otsuka Pharmaceutical Tokushima research institute. Thirty rats of each strain were divided into three groups: the glucose, the vitamin E, and the guava juice ingestion groups. The 100% guava juice (Okinawa Bireleys Inc., Okinawa, Japan) was diluted with distilled water. 25% guava juice (0.4 mg/ml guava polyphenol content) was used for the ingestion experiment. Vitamin E suspension (0.8 mg/ml) was prepared by dissolving 0.1 ml (95 mg vitamin E) of vitamin E (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in 2 mg/ml sodium deoxycholate (Wako Pure Chemical Industries, Ltd.). Control rats were ingested with the glucose solution (4 mg/ml), the concentration of which was adjusted to that of 25% guava juice. These test solutions were given *ad libitum*. The ingestion of these test solutions was continued from 9 to 32 weeks old. All rats were bred with Rodent Diet CE2 solid food (Japanese Clare Inc., Tokyo, Japan). The breeding conditions of rats in the experiment period were:

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room temperature at 23 ± 1°C, humidity at 60 ± 5%, and 12 hr of the day-and-night cycle (day time: 8:30–20:30).

Measurement of Serum Lipid Parameters ——— The serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL)-cholesterol, and free fatty acid (FFA) concentrations were measured with Hitachi-7170 automatic analysis equipment using measurement reagent TCHO-L®, TG-L®, HDL-L®, and NEFA® (Serotec Inc., Sapporo, Japan).

Measurement of Blood Glucose Level and Plasma Insulin Concentration ——— The oral glucose tolerance test (OGTT) was performed according to the method of Luan *et al.*[4] After 16 hr-fasting, rat's tail vein was punctured with 26-gauge needle before and 30, 60, and 120 min after ingestion of glucose solution (0.25 g/ml) as much as 2 g glucose/kg body weight, and then blood was collected with heparinized capillary tubes (Drummond Scientific Co., Pennsylvania, U.S.A.). Blood glucose level was measured using MediSense® Precision Xtra (Abbott Japan, Tokyo, Japan). Rat plasma insulin concentration was measured using the ELISA Insulin Kit (Morinaga Institute of Biological Sciences, Yokohama, Japan).

Statistical Analysis ——— StatView version 5 (SAS Institute Inc., North Carolina, U.S.A.) was used for statistical analysis. Two-way repeated-measures analysis of variance (ANOVA) was performed for comparison of time course changes in the means. The contrast method was used for a multiple comparison.

RESULTS

Effect of Long-Term Ingestion of Guava Juice on Body Weight, Food Intake and Drink Intake

The body weights of both OLETF and LETO rats were increased with age. Although the body weight of OLETF rats was increased more significantly than that of LETO rats after 8-weeks old, there was no significant difference among the groups in OLETF rats (Fig. 1). The amounts of food intake in all the three groups of OLETF rats were significantly increased as compared with that of LETO rats (glucose). However, there was no significant difference among the groups in OLETF rats (data not shown). The weekly-averaged quantities of drinking glucose, vitamin E and guava juice in both OLETF and LETO rats were almost constant throughout the experiment period. The averaged daily intake of vitamin E was 32 mg and guava polyphenol was 16.8 mg in OLETF rats. In LETO rats, vitamin E was 28.8 mg and guava polyphenol was 16 mg.

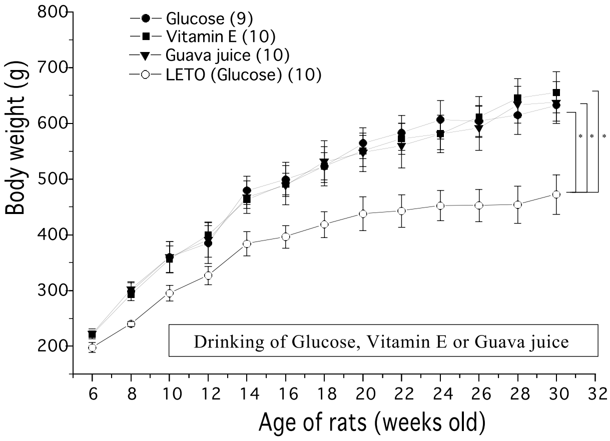

Fig. 1. Time Course of Changes in Body Weight of OLETF and LETO Rats

The data of weekly-averaged body weight of rats were plotted to age of rats. ○: the glucose group of LETO rats ingesting glucose solution (4 mg/ml), ●: the glucose group of OLETF rats ingesting glucose solution (4 mg/ml), ■: vitamin E group of OLETF rats ingesting vitamin E suspension (0.8 mg/ml), ▼: guava juice group of OLETF rats ingesting 25% guava juice. Data represents means ± S.D. $*p < 0.05$ *vs.* the glucose group of LETO rats.

Effect of Ingestion of Guava Juice on the Levels of Serum Lipids

TC, TG and FFA levels were increased with age, and these values in all the three groups of OLETF rats were significantly increased at 4 months after the start of ingestion as compared with LETO rats (Fig. 2A–2C). However, HDL level was not changed (Fig. 2D). In the OLETF rats, TC level in the guava juice group had a tendency to decrease, whereas TG level had a tendency to increase as compared with the control group.

Effect of Long-Term Ingestion of Guava Juice on the Glucose Metabolism

OGTT of 32-weeks old rats revealed that fasting blood glucose level in all the three groups of OLETF rats did not differ with that of the glucose group of LETO rats (at Pre in Fig. 3A). In the OLETF rats, the fasting blood glucose levels were not changed by the ingestion of vitamin E or guava juice. However, the blood glucose levels in all the three groups of OLETF rats were significantly increased at 30, 60, and 120 min after the ingestion of glucose as compared with those of LETO rats (Fig. 3A). Thus, all the three groups of OLETF rats had shown

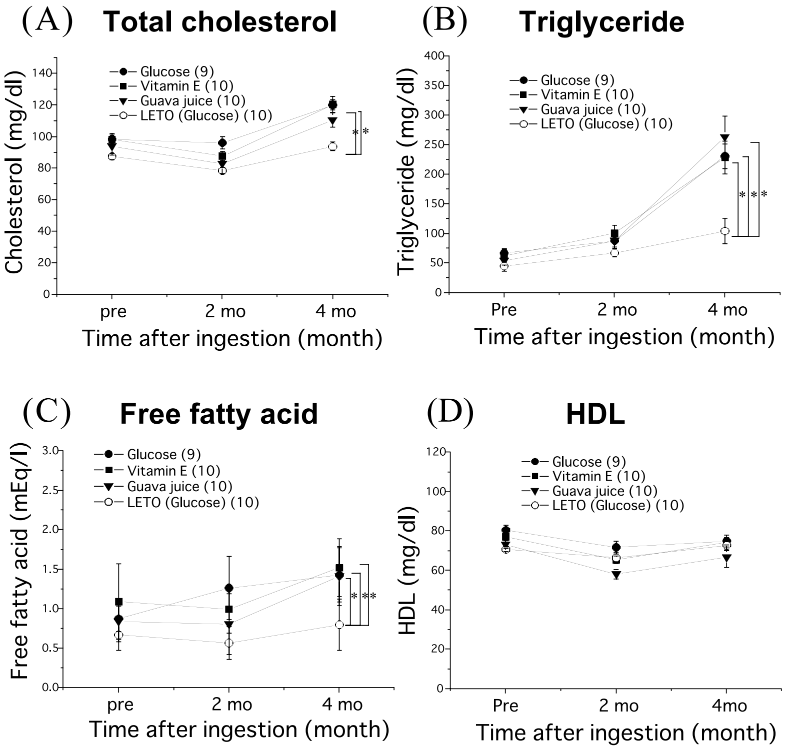

Fig. 2. Effect of Long-Term Ingestion of Glucose, Vitamin E, and Guava Juice on The Serum Lipid Measures

(A): Total cholesterol, (B): Triglyceride, (C): Free fatty acid, and (D): HDL-cholesterol. Blood was sampled from rat's tail vein at any time without fasting, and then the serum concentrations of total cholesterol, triglyceride, free fatty acid, and HDL were measured. The collected data was divided into three categories based on the length of ingestion period: pre (from 6 to 8-weeks old rats, before ingestion), 2 mo (from 10 to15-weeks old rats) and 4 mo (from 20 to 28-weeks old). ○: the glucose group of LETO rat ingesting glucose solution (4 mg/ml), ●: the glucose group of OLETF rats ingesting glucose solution (4 mg/ml), ■: vitamin E group of OLETF rats ingesting vitamin E suspension (0.8 mg/ml), ▼: guava juice group of OLETF rats ingesting 25% guava juice. Data represents means ± S.E. *$p < 0.05$ *vs*. the glucose group of LETO rats. The numbers in parenthesis are the number of rats tested.

the characteristics of diabetes. In the OLETF rats, the blood glucose level of the guava juice group showed a tendency to increase at 30 min and to decrease at 60 and 120 min, as compared with the glucose group (Fig. 3A). Measurement of plasma insulin concentration revealed that insulin concentrations in the guava juice group were significantly increased before and 30, 60, and 120 min after the ingestion of glucose as compared with those of the glucose groups of OLETF and LETO rats (Fig. 3C). To test if the guava juice ingestion increased plasma insulin concentration, OGTT was performed 10 weeks after discontinue of the ingestion of guava juice. Consequently, blood glucose levels in the glucose and the guava juice groups of OLETF rats were significantly increased as compared with the glucose group of LETO rats (Fig. 3B). However, there was no significant difference of blood glucose level between the glucose and the guava juice groups of OLETF rats. Moreover, plasma insulin concentration in the guava juice goup of OLETF rats did not differ with that of the glucose group of OLETF rats (Fig. 3D).

DISCUSSION

In the present study, we demonstrated that the OLETF rats had developed obesity due to the increase in the amount of food intake (Fig 1). There were no significant differences of the body weight and serum TC and TG concentrations among the groups of OLETF rats. Therefore, the ingestion of guava juice had no effect on the overweight control.

Since the blood glucose levels at 30, 60, and 120 min of OGTT in 32-weeks old OLETF rats were remarkably increased, all the three groups of OLETF rats were considered to have developed diabetes mellitus (Fig. 3A). Although the blood glucose level in the guava juice group of OLETF rats was not changed as compared with the glucose group, the amount of initial insulin secretion was significantly

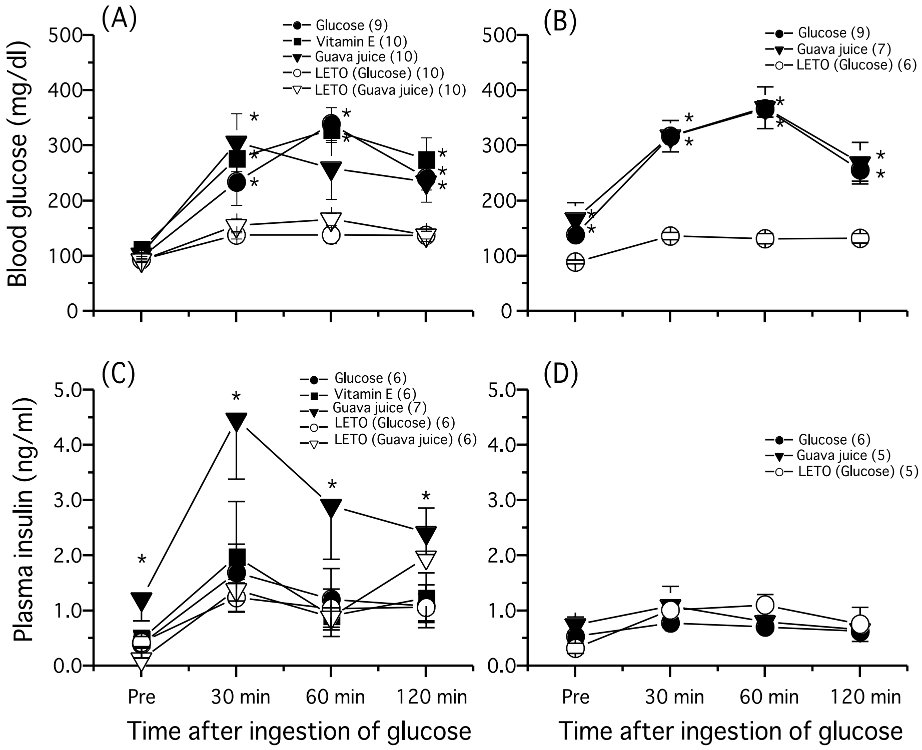

Fig. 3. Time Courses of Changes in Blood Glucose Level and Plasma Insulin Concentration

Blood glucose level (A and B) and plasma insulin concentration (C and D) were measured on oral glucose tolerance test (OGTT) in 32-weeks old OLETF and LETO rats (A and C) and in 42-weeks old OLETF and LETO rats at 10 weeks after discontinue of ingestion of guava juice (B and D). Blood was sampled from the tail vein before and 30, 60, and 120 min after ingestion of glucose (2 g/kg body weight). ○: the glucose group of LETO rat ingesting glucose solution (4 mg/ml), ▽: the guava juice group of LETO rats ingesting 25% guava juice, ●: the glucose group of OLETF rats ingesting glucose solution (4 mg/ml), ■: vitamin E group of OLETF rats ingesting vitamin E suspension (0.8 mg/ml), ▼: the guava juice group of OLETF rats ingesting 25% guava juice. Data represents mean ± S.E. *$p < 0.05$ and **$p < 0.01$ *vs*. the glucose group of LETO rats. The numbers in parenthesis are the number of rats tested.

increased. However, insulin concentrations were not changed in the guava juice ingestion group of LETO rats (Fig. 3C). Furthermore, plasma insulin concentration in the guava juice ingestion group of OLETF rats was restored to the level of glucose group of OLETF rats after 10-weeks discontinue of the ingestion of guava juice (Fig. 3D). Taken together, the guava juice ingestion might enhance the basal secretion of insulin as well as the insulin secretion in response to the rise in blood glucose level especially in OLETF rats.

The insulin secretion might be stimulated in OLETF rats by other factors rather than the rise in blood glucose level. It is known that long chain FFA stimulates insulin secretion. Although serum TG concentration of the guava juice group of OLETF rats was slightly increased, serum FFA concentration was not changed at 4 months after the start of ingestion (Fig. 2). Therefore, FFA might not be involved in the elevated insulin concentration in the guava juice group. It has been known that dietary fiber and polyphenol are abundantly contained in guava juice and that guava polyphenol has an anti-oxidization action.[3] It needs to be clarified which ingredient in guava juice acts to stimulate insulin secretion in OLETF rats.

Moreover, the long-term ingestion of vitamin E or guava juice did not alter glucose metabolism such as glucose consumption in the peripheral tissue and the glucose absorption from an intestinal tract. The ingestion of guava juice in OLETF rats 30 min prior to OGTT did not decrease the blood glucose level as compared to the ingestion of glucose in OLETF rats (data not shown). This indicates that the guava juice does not inhibit glucose absorption from the intestinal tract and not inhibit the enzyme activity for carbohydrate digestion in the intestinal tract. In agreement, Tamura *et al*. reported that the extracts from dried guava fruit did not decrease the glucose level of OGTT, but did improve glucose tolerance in Wistar rats.[5]

For comparison we used vitamin E as another antioxidant in this study. Although vitamin E had no effect on the glucose and insulin levels of OGTT (Fig. 3), there might be effective on reducing the oxidants such as low-density lipoprotein.[6)]

In the present study, we investigated whether the long-term ingestion of guava juice had anti-diabetic and anti-obese effects using spontaneous NIDDM model OLETF rats. Although the long-term ingestion of guava juice did not inhibit the development of obese, the blood glucose level at 60 and 120 min for OGTT showed a tendency to decrease in OLETF rats. The decrease in blood glucose level might be produced by the stimulation of insulin secretion by the long-term ingestion of guava juice. Identification of the substance in guava juice, which stimulates insulin secretion, needs to be done in a future study.

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