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NOTE

Changes in Plasma Glucose in Otsuka Long-Evans Tokushima Fatty Rats After Oral Administration of Maple Syrup

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Abstract: We investigate whether maple syrup is a suitable sweetener in the management of type 2 diabetes using the Otsuka Long-Evans Tokushima Fatty (OLETF) rat. The enhancement in plasma glucose (PG) and glucose absorption in the small intestine were lower after the oral administration of maple syrup than after sucrose administration in OLETF rats, and no significant differences were observed in insulin levels. These data suggested that maple syrup might inhibit the absorption of glucose from the small intestine and preventing the enhancement of PG in OLETF rats. Therefore, maple syrup might help in the prevention of type 2 diabetes.

Key words: maple syrup, plasma glucose, grade, type 2 diabetes mellitus, OGT test

1 INTRODUCTION

Individuals with metabolic syndrome are at increased risk of developing type 2 diabetes mellitus and cardiovascular disease, as well as increased mortality caused by cardiovascular diseases and all other causes[1, 2]. The prevalence of type 2 diabetes mellitus resulting from metabolic syndrome is increasing rapidly, and is affecting the health of millions of humans, and will continue to do so in the near future. The restriction of sweeteners, such as sucrose, maple syrup, honey, etc., is important to prevent obesity and the promotion of diabetes mellitus, and the identification of sweeteners with lower glycemic indices is expected to improve quality of life for patients with type 2 diabetes mellitus.

Maple syrup is a natural sweeter consumed by many people of all ages throughout the world. Maple syrup generally contain sucrose as main content, several percent of glucose and trace amounts of oligosaccharides, and have around pH 6.0 to 6.5[3]. Apart from sucrose, which is its dominant sugar, maple syrup contains organic acids, amino acids, vitamins, and minerals including manganese and zinc[3–7]. Maple syrup is obtained by using heat to evaporate maple sap collected from maple trees during the spring season; the characteristic flavor, color, and odor of maple syrup develop during the concentration process of transforming sap to syrup. The color of the syrup typically becomes darker as the season progresses and antioxidant activity is proportional to the darkening color of the maple syrup[8]. Based on United States and Canadian standards, maple syrup is classified into five grades: grade AA (extra light), grade A (light), grade B (medium), grade C (amber), and grade D (dark)[8].

Recent studies have shown that maple syrup contains phenolic compounds such as lignans, as well as coumarin[9, 10], quebecol[11], and ginnalin[12, 13]. In addition, the phenolic compounds in maple syrup may possess antioxidant activity[8], and an in vitro study of a butanol extract of maple syrup demonstrated inhibitory activity toward α-glucosidase[14]. We also reported that the increase in plasma glucose (PG) is lower after the oral administration of maple syrup that has been prepared by dissolving maple sugar in water than after the administration of sucrose in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a model of type 2 diabetes mellitus[15, 16]. These data suggest the possibility that maple syrup might be used to prevent obesity and the development of diabetes mellitus compared

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with other major sweeteners, such as sucrose and glucose-fructose syrup. However, there are almost no reports or scientific evidence to indicate the effects of the oral consumption of various maple syrup grades on PG levels. Therefore, there is a need to evaluate possible differences in maple syrup grade as a part of evaluating maple syrup for use as a sweetener in the management of diabetes mellitus. In this study, we administered three types of maple syrup, classified by color, to OLETF rats in order to investigate whether maple syrup is suitable as a sweetener in the management of type 2 diabetes.

2 EXPERIMENTAL

2.1 Materials and Animals

Maple syrups were purchased at a local grocery. We chose three maple syrups of different colors, and classified these syrups as three types based on increasingly deepening color: Maple syrup (I) is slightly golden; Maple syrup (II) is amber; and Maple syrup (III) is very dark brown.

Male 7-week-old Wistar rats (Kiwa Laboratory Animals Co., Ltd.) and 40-week-old OLETF rats (Otsuka Pharmaceutical Co., Ltd.) were used in this study. All procedures were performed in accordance with the guidelines of the Kinki University Faculty of Pharmacy Committee for the Care and Use of Laboratory Animals.

2.2 Measurement of Body Weight and Blood Tests for Diabetes Mellitus

Body weights and some blood test parameters for diabetes mellitus were measured for rats at 40 weeks of age. After a 15-h fast, blood was drawn from the tail vein of each rat without anesthesia, and PG, triglycerides (TG), total cholesterol, and insulin levels were measured using the Glucose Assay Kit (BioVision Inc, Milpitas, Canada), Accutrend GCT (Roche Diagnostics, Mannheim, Germany), a Cholesterol E-Test Kit (Wako, Osaka, Japan) and an ELISA Insulin Kit (Morinaga Institute of Biological Science Inc., Kanagawa, Japan), respectively.

2.3 Oral Glucose Tolerance (OGT) Test Using Sucrose and Maple Syrup

Rats were fasted for 15 h, and sucrose or maple syrup (an amount equivalent to 1.5 g sucrose/kg rat) was administered orally to each rat. Blood samples were taken from the tail veins at 0 (just before glucose administration), 30, 60, 120, and 180 min. PG and insulin levels were determined.

We analyzed the differences in PG (ΔC_{PG}, mg/dl) and insulin ($\Delta C_{insulin}$, ng/dl) concentrations between OLETF rats with or without the oral administration of sucrose or maple syrup. The area under the curve for $\Delta C_{PG\ or\ insulin}$ ($AUC_{0\text{-}180\ min}$) was calculated according to the following equation:

$$AUC_{0\text{-}180min} = \int_{0min}^{180min} \Delta C_{PG\ or\ insulin}\, dt \qquad \text{Eq. 1}$$

Briefly, t is time (min) after the oral administration of sucrose or maple syrup. $AUC_{0\text{-}180\ min}$ was determined according to the trapezoidal rule up to 180 min, which was the last point at which PG or insulin concentration was measured.

2.4 Assay of Sucrose and Maple Syrup Absorption Using *In Situ* Loop Technique

Seven-week-old Wistar rats were fasted overnight and anesthetized with pentobarbital (30 mg/kg). A small midline incision allowed the gentle exposure of a 4-cm to 5-cm target portion of intestine. This target small intestine, selected because of its suitable vasculature to collect venous blood, was washed gently with saline solution. An 8-cm length of silicon tubing (outer diameter: 2.0 mm; inner diameter: 1.0 mm; TERUMO Corp., Tokyo, Japan) was inserted into one end of the intestine and tied securely with a surgical suture. The opposite end was tied and 1.0 ml of sucrose or maple syrup (an amount equivalent to 1.5 g sucrose/kg rat) was injected into the intestine through the tube. Heparin (10 mg/kg) was injected into the femoral vein. The mesenteric vein was cannulated with an appropriate size of polyethylene tubing (Hibiki Co., Tokyo, Japan), and all venous blood was collected in a microtube. This blood was centrifuged at 10,000 rpm for 30 min at 4℃, and the PG levels in the obtained serum were determined.

2.5 Statistical Analysis

All data are expressed as the mean ± standard error (S.E.) of the mean. Unpaired Student's or Aspin-Welch's t-test was used to evaluate statistical difference. P values less than 0.05 were considered statistically significant.

3 RESULTS

3.1 Changes in Plasma Glucose Levels in 40-Week-Old OLETF Rats After the Oral Administration of Three Types of Maple Syrup

All factors in this study were significantly higher for OLETF rats than for age-matched control rats (Long-Evans Tokushima Otsuka rats, LETO rats) (Table 1). In addition, the OLETF rats had developed type 2 diabetes mellitus with metabolic syndrome. The OLETF rats were randomly divided into four groups; food intake, water intake, body weight, PG, TG, total cholesterol, and insulin levels were similar among the four groups (Table 2). Three of the four groups were each administered a specific type of maple syrup [Maple syrup (I), Maple syrup (II), or Maple syrup (III)], and sucrose was administered to the remaining group. The different types of maple syrup resulted in sig-

Table 1 Concentration of Sucrose in Maple Syrup and Maple Syrup Dose to OLETF Rats in This Study.

	Sucrose (g/100g syrup)	Dose (g maple syrup/kg rat)
Maple Syrup (I)	57.5	2.66
Maple Syrup (II)	58.7	2.55
Maple Syrup (III)	56.4	2.61

Table 2 Food Intake, Water Intake, and Blood Test Values in OLETF and LETO Rats in This Study.

	OLETF (n=12)	LETO (n=5)
Food intake (g/day/rat)	39.7 ± 0.83	25.9 ± 3.7
Water intake (ml/day/rat)	80.9 ± 1.5	41.5 ± 2.8
Body weight (g)	655.8 ± 4.1	470.1 ± 15.3
PG (mg/dl)	250.4 ± 9.7	110.3 ± 9.8
TG (mg/dl)	389.8 ± 5.9	120.1 ± 10.0
Total cholesterol (mg/dl)	221.6 ± 4.0	101.2 ± 16.1
Insulin (ng/dl)	315.7 ± 5.8	109.6 ± 19.7

Data are presented as mean ± S.E.

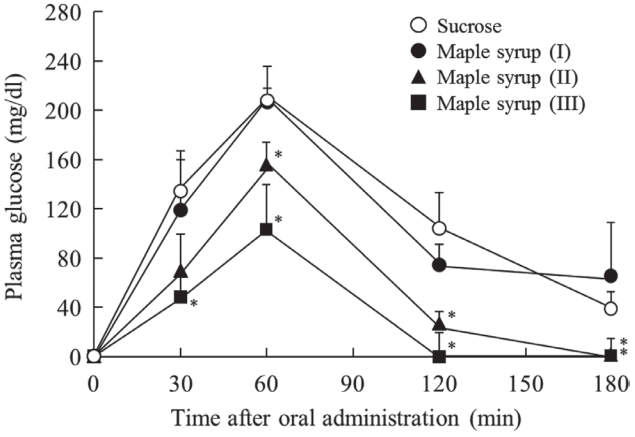

Fig. 1 Changes in plasma glucose levels in 40-week-old OLETF rats after the oral administration of sucrose (open circles) or maple syrup types I (closed circles), II (closed triangles), or III (closed squares). Data are presented as mean ± S.E. of 3 independent rats. * $p < 0.05$ vs. OLETF rats that were administered sucrose for each category.

nificantly different PG levels at respective time points (Fig. 1). The PG levels of all OLETF rats peaked 60 min after the oral administration of sucrose or maple syrup, and then gradually decreased (Fig. 1). At 2 h after the administration of Maple syrup (II) or Maple syrup (III), the OLETF rats' PG levels were similar to pre-prandial levels. In contrast, at 3 h after the administration of Maple syrup (I) or sucrose the OLETF rats' PG levels remained significantly above pre-prandial levels. Table 4 shows the $AUC_{0\text{-}180\ min}$ for PG levels in OLETF rats administered sucrose versus the three types of maple syrup. OLETF rats administered Maple syrup (II) or Maple syrup (III) exhibited lower PG enhancement and lower peak PG than OLETF rats administered sucrose (Fig. 1); the $AUC_{0\text{-}180\ min}$ values for PG in OLETF rats administered Maple syrup (II) and Maple syrup (III) were 60.1% and 42.9% of the respective value for OLETF rats that had been administered sucrose (Table 3).

3.2 Comparison of the Enhancement of Plasma Glucose Levels in 40-Week-Old OLETF Rats After the Oral Administration of Sucrose vs. Maple Syrup

The insulin levels of OLETF rats peaked 30 min after the oral administration of either sucrose or maple syrup, and enhanced insulin levels were observed as late as 3 h after the oral administration (Fig. 2). $AUC_{0\text{-}180\ min}$ indicated that no significant differences in plasma insulin levels were observed between OLETF rats administered sucrose versus maple syrup (Table 3). Figure 3 shows the glucose absorption from the small intestine into the bloodstream, after injections of sucrose and the three types of maple syrup using the *in situ* loop technique. PG increased for the first 30 min after the injection of sucrose or maple syrup. The PG level was approximately 350 mg/dl at 30 min after the injection of sucrose, and sucrose and Maple syrup (I) each caused similar changes in PG level. In contrast, the changes of PG levels after the injection of Maple syrup (II) and Maple syrup (III) were significantly lower than that of sucrose.

4 DISCUSSION

In work such as this study, in which we endeavored to accurately determine the effects of the oral administration of sucrose and various maple syrup grades on PG levels in a diabetes model, the selection of the experimental animal is very important. The OLETF rat is an established model of human type 2 diabetes mellitus[15] in which nearly 100%

Table 3 Food Intake, Water Intake, and Blood Test Values for Diabetes Mellitus in OLETF Rats in This Study.

	Sucrose	Maple Syrup		
		I	II	III
Food intake (g/day/rat)	38.3 ± 1.9	38.9 ± 2.0	40.1 ± 2.1	39.1 ± 1.7
Water intake (ml/day/rat)	81.3 ± 3.8	80.1 ± 3.2	81.0 ± 3.0	80.9 ± 3.0
Body weight (g)	653.3 ± 3.3	653.5 ± 8.8	693.3 ± 43.3	643.1 ± 6.7
PG (mg/dl)	254.9 ± 12.9	241.1 ± 40.1	285.0 ± 18.5	240.1 ± 27.1
TG (mg/dl)	381.6 ± 15.2	376.3 ± 13.9	390.0 ± 17.8	383.5 ± 12.7
Total cholesterol (mg/dl)	214.1 ± 9.7	217.0 ± 9.4	219.6 ± 9.1	215.3 ± 9.3
Insulin (ng/dl)	317.2 ± 15.8	316.5 ± 11.4	313.6 ± 9.9	315.3 ± 16.1

Data are presented as mean ± S.E. of 3 independent OLETF rats.

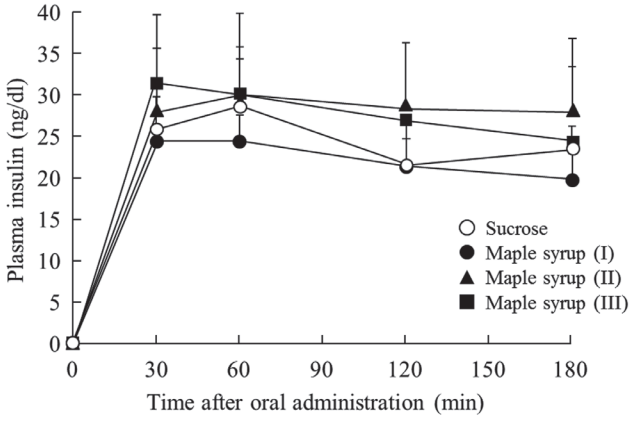

Fig. 2 Changes in plasma insulin levels in 40-week-old OLETF rats after the oral administration of sucrose (open circles) or maple syrup types I (closed circles), II (closed triangles), or III (closed squares). Data are presented as mean ± S.E. of 3 independent rats.

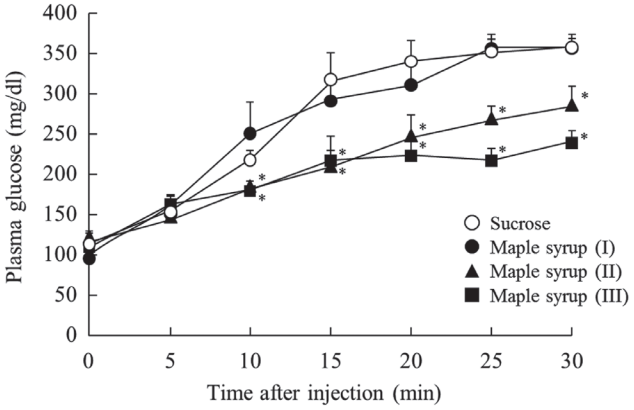

Fig. 3 Glucose absorption in 7-week-old Wistar rats after the injection of sucrose (open circles) or maple syrup types I (closed circles), II (closed triangles), or III (closed squares) using the in situ loop technique. Wistar rats (7 weeks old) were anesthetized with pentobarbital (30 mg/kg), and sucrose or maple syrup (an amount equivalent to 1.5 g sucrose/kg rat weight) was injected into the small intestine. Data are presented as mean ± S.E. of 3 independent rats. * $p < 0.05$ vs. sucrose-injected rats for each category.

of males develop a diabetic syndrome by 25 weeks of age. OLETF rats exhibit higher food intake and water consumption, and their body weight and TG levels are significantly increased compared with the control rat type.

In this study, we use three types of maple syrup. The concentration of sucrose, main content of maple syrup, did not differ significantly among the three types of maple syrup (data not shown). The obtained results by this study showed that changes in insulin level did not correlate with the reduced enhancement of PG in OLETF rats after the administration of Maple syrup (II) and Maple syrup (III). Apostolidis *et al.*[14] reported previously that maple syrup has in vitro α-glucosidase inhibitory activity, and α-glucosidase in epithelial cells of the small intestine decomposes disaccharides such as sucrose to glucose. Thus, we used the in situ loop technique to investigate glucose absorption from the small intestine into the bloodstream after injections of sucrose or maple syrup. The results suggest that the inhibitory effect of maple syrup administration on the enhancement of PG might be to suppress the absorption ratio of glucose from the small intestine, and the stronger inhibitory effect might be associated with darker maple syrup grade.

These data also suggest that maple syrup might contain some useful compound, such as a novel α-glucosidase inhibitor, and the darker-colored maple syrup might contain more of this compound than the lighter-colored syrup. Additional studies are needed to identify the compounds responsible for the observed low glucose absorption after the

Table 4 $AUC_{0\text{-}180\ min}$ for Plasma Glucose and Insulin in 40-Week-Old OLETF Rats After the Oral Administration of Sucrose or Maple Syrup Types I-III.

	Sucrose	Maple Syrup		
		I	II	III
PG (mg・min/dl)	16427 ± 1892	15333 ± 3037	10001 ± 042*	7040 ± 1957*
Insulin (ng・min/dl)	2689 ± 376	2475 ± 1282	3041 ± 494	2932 ± 292

The *AUC* were estimated using Eq. 1. Data are presented as mean ± S.E. of 3 independent rats.
* $p < 0.05$ vs. sucrose for each category.

oral administration of maple syrup.

These findings provide significant information concerning the potential for the use of maple syrup as a sweetener in the management of type 2 diabetes owing to its lower glycemic index, and indicate that maple syrup, especially darker-colored maple syrup, should be useful in the management of type 2 diabetes.

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