Adverse effects of fructose-containing beverages on the health for young women

果糖含有飲料が若年女性の健康に及ぼす影響

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Adverse effects of fructose-containing beverages on the health for young women

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General introduction

Triglyceride-rich lipoprotein (TRL) in plasma is known to contain chylomicron (CM), very low-density lipoprotein (VLDL) and their remnants. TRL increases in the postprandial state and postprandial hyperlipidemia is known to be a major risk factor for coronary heart disease (CHD) as reported by Zilversmit in 1979[1)]. Increased production of TRL is caused by high dietary fat intake, and enhanced biosynthesis of the TG and apoB in the liver and increased FFA flux from adipose tissue in insulin resistant states.

Following the ingestion of meal, CM derived from the intestine is hydrolyzed by the lipolytic enzyme, lipoprotein lipase (LPL), which is anchored on the capillary endothelium of extrahepatic tissues, particularly adipose tissue and skeletal muscle[2)], and converted to CM-remnant (CM-R). This is called the exogenous lipoprotein pathway. In an another pathway, namely endogenous lipoprotein pathway, VLDL derived from the liver is hydrolyzed by the same lipolytic enzyme, LPL[2)], and converted to VLDL-R. TG in VLDL-R is hydrolyzed by hepatic lipase and metabolized to LDL. In addition, the transfer of TG from VLDL, VLDL-R and LDL for CE on HDL by the reverse cholesterol transport. TG-rich VLDL particle leads to TG-enrichment and cholesteryl-ester depletion of produced LDL and HDL particles, therefore increasing potentially atherogenic small dense LDL and small HDL particles. Smaller HDL particles are more likely to be excreted by the kidney, causing the low HDL-C levels in plasma[3)].

Our laboratory has studied postprandial lipoprotein metabolism after fat load in lean and obese young men, middle-aged and young women. Those results showed that postprandial increase of TRL may be more important than the fasting TG level[4-6)]. It has also been shown that fasting apolipoprotein B-48 (apoB48) levels are raised in individuals with metabolic syndrome or with high fasting plasma TG levels[7, 8)].

In the USA, high fructose corn syrup (HFCS) was developed industrially as an alternative to sucrose over the period from 1970 to 1985[9)]. In addition, many studies have shown a relationship between the intake of sugar-sweetened beverages and weight gain[10)]. Overconsumption of fructose can have adverse effects on human health. Diets containing >15% of energy derived from fructose, as compared with glucose, are consistently associated with increases in both fasting and postprandial triglyceride (TG) concentrations[11, 12)]. Similarly, the consumption of HFCS as an

alternative to sucrose has been increasing in Japan since 1980. Soft drinks account for approximately 50% of the total consumption of HFCS[13]. However, the influence of fructose intake on carbohydrate and lipid metabolism remains to be clarified and very little information about the effects of fructose comsumption on the Japanese subjects is available. Futhermore, overconsumption of fructose can have other adverse effects on human health. It has also been reported that large amounts of fructose often induce gastrointestinal symptoms, such as bloating, abdominal pain, and diarrhea[14, 15].

The highest consumers of fructose are adolescents and young adults in the USA[16], and this is probably also the case in Japan. Therefore, we enrolled young healthy Japanese women as subjects in this study. At first, we examined the effect of the ingestion of fructose and HFCS on particularly postprandial lipid metabolism. Next, we investigated the absorption of a fructose-containing beverages by measuring the concentration of breath hydrogen after the intake of fructose-containing beverages.

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Chapter 1

Simultaneous ingestion of fructose with fat exacerbates postprandial exogenous lipidemia in young healthy Japanese women

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Abstract

Aim: To investigate the acute effects of simultaneous ingestion of fructose and fat on postprandial lipoprotein metabolism in young healthy women.

Methods: Nine young healthy Japanese women with a normal weight (body mass index: 18.5≤–<25 kg/m^2), a normal ovarian cycle and an apolipoprotein E 3/3 phenotype were enrolled as participants and studied on four occasions. At each session, the subjects ingested one of four beverages containing either glucose or fructose (0.5 g/kg body weight each) with or without OFTT cream (1 g/kg, 0.35 g/kg as fat) in a randomized crossover design. Blood samples were collected at baseline and 0.5, 1, 2, 4 and 6 hours after ingestion.

Results: The ingestion of fructose combined with fat led to significantly higher rise in the serum triglyceride (TG), remnant-like particle (RLP)-TG, remnant lipoprotein-cholesterol (RemL-C) and apolipoprotein B-48 (apoB48) concentrations with delayed peaks compared with that observed following ingestion of the other three types of beverages. The incremental area under the curve (ΔAUC)-TG and ΔAUC-apoB48 were larger than those observed for the ingestion of fat only. The serum RLP-TG and apoB48 concentrations returned to the fasting levels (0 hours) at the end of the test (6 hours) following the ingestion of fat only; however, these concentrations did not return to the fasting levels following the intake of fructose combined with fat.

Conclusions: These findings suggest a delay in the clearance of intestinal TG-rich lipoproteins, namely chylomicron and its remnant, following the ingestion of fructose combined with fat. The simultaneous ingestion of fructose and fat markedly enhances postprandial exogenous lipidemia in young healthy Japanese women.

Introduction

High fructose corn syrup (HFCS) was developed industrially as an alternative to sucrose in the USA over the period from 1970 to 1985[1]. In Japan, the consumption of HFCS has been increasing since 1980, while that of sucrose has decreased[2], and HFCS currently accounts for approximately 40% of the total consumption of sugar, ranking just behind the USA[3]. The products in which HFCS is used include soft drinks, which account for approximately 50% of the total consumption of HFCS[2]. However, detailed research has not yet been conducted on the daily consumption of HFCS and fructose by Japanese people.

In the USA, many studies have shown a relationship between the intake of sugar-sweetened beverages and weight gain[4], and exaggerated postprandial triglyceride (TG) response observed following the ingestion of HFCS has been shown to be caused by the fructose component[5]. Some studies have shown that the plasma concentration of TG increases significantly when fructose is ingested simultaneously with fat compared to that observed following fat intake only[6, 7]. Therefore, simultaneous ingestion of fructose and fat may possibly exacerbate postprandial lipidemia. The levels of triglyceride-rich lipoproteins (TRL), namely, chylomicrons (CM), very low-density lipoproteins (VLDL) and their remnants, increase in the postprandial state, and postprandial hyperlipidemia is known to be a major risk factor for coronary heart disease, as proposed by Zilversmit[8].

However, the influence of fructose intake on carbohydrate and lipid metabolism remains to be clarified, and very little information about the effects of fructose comsumption on the Japanese subjects is available. The World Health Organization has proposed that added sugars should provide no more than 10% of dietary energy[9]. At present, there are no evidence-based regarding the upper limits of the intake of fructose, HFCS and sucrose for maintaining health in Japanese individuals[10]. The consumers with the highest intake of fructose in the USA are adolescents and young adults[11], which is most likely also the case in Japan. Therefore, we enrolled young healthy Japanese women as subjects and examined the acute effects of fructose consumption on postprandial lipidemia.

Aim

To elucidate the effects of simultaneous ingestion of fructose and fat on postprandial lipoprotein metabolism in young healthy Japanese women.

Methods

Subjects

Nine young healthy Japanese women with a normal weight, a normal ovarian cycle and an apoE phenotype 3/3 were enrolled as participants. A normal weight was defined as a body mass index (BMI) of ≥18.5 kg/m^2 and <25 kg/m^2. All of the subjects were nonsmokers, had no apparent acute or chronic illnesses, and were not taking any medications or dietary supplements. This study was approved by the Institutional Review Board of the Sugiyama Jogakuen University School of Life Studies, and each subject provided their written informed consent for study participation.

Anthropometric and Body Composition Measurement

Body weight and height were measured according to standard methods. Waist circumference was assessed as the abdominal girth at the level of the umbilicus, and the hip circumference was measured at the level of the greater trochanters. The waist-to-hip (W/H) ratio was calculated. Body composition, including the visceral fat area (VFA) was analyzed using an eight-polar bioelectrical impedance method, with the InBody720 (BioSpace, Tokyo, Japan).

Fructose, Glucose, and Fat Load Test

Each subject was studied on four occasions. At each test trial, the subjects ingested one of four beverages containing either fructose or glucose (0.5 g/kg body weight each) with or without oral fat tolerance test (OFTT) cream (Jomo, Takasaki, Japan; 1 g/kg, 0.35 g/kg as fat) in a randomized crossover design. OFTT cream was used as previously described[12-14]. The four beverages were prepared as follows: Fru: 0.5 g/kg of fructose (Nisshin Seito, Tokyo, Japan) mixed with no-sugar soda (Suntory, Tokyo, Japan), that is 1 g/10 mL as fructose; Fat: 1 g/kg of OFTT cream; Fru+Fat: fructose (0.5 g/kg) mixed with no-sugar soda supplemented with OFTT cream (1 g/kg), that is 1 g/10 mL as fructose; Glu: 0.5 g/kg of glucose (1.5 mL/kg of Trelan-G75™, 1 g/3 mL as glucose, Ajinomoto, Tokyo, Japan). All beverages were used at a concentration of 10% (w/v).

Experimental Design

One of the four beverages mentioned above was administered after a 12-hour overnight fast. The subjects abstained from consuming caffeine or alcohol on the day before the experiment. Venous blood samples were obtained before (0 hours)

and 0.5, 1, 2, 4 and 6 hours after ingestion. During the test, the subjects avoided exercise and eating, but had free access to water after 1 hour. All blood samples were obtained in the supine position. The experiments were conducted at least four weeks apart. There was an interval of four weeks between the test days to minimize the confounding effects of the subjects' menstrual status on lipid metabolism.

Biochemical Analysis

The serum samples were immediately refrigerated (4°C) or frozen (−80°C) until the analysis. Fructose was measured enzymatically (BioAssay Systems, CA, USA). Glucose was measured using a mutarotase GOD method (Wako, Osaka, Japan). Insulin was measured using a chemiluminescent enzyme immunoassay (Fujirebio, Tokyo, Japan). Insulin resistance was evaluated according to the homeostasis model assessment for insulin resistance (HOMA-IR) [15]. The hemoglobin A1c (HbA1c) level was measured using a latex agglutination method (Fujirebio) and expressed as the National Glycohemoglobin Standardization Program (NGSP) value. The levels of free fatty acids (FFA) (Eiken Chemical, Tokyo, Japan) and lactate (Kyowa Medex, Tokyo, Japan) were measured enzymatically.

The level of total cholesterol (TC) was measured enzymatically (Sysmex, Hyogo, Japan). The level of high-density lipoprotein cholesterol (HDL-C) was measured using a direct method (Fujirebio). The level of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. The level of small, dense LDL-C (sdLDL-C) was measured enzymatically (Denka Seiken, Tokyo, Japan). The TG level was measured enzymatically (Sekisui Medical, Tokyo, Japan). The remnant-like particle-TG (RLP-TG) level was measured using an immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan). The remnant lipoprotein cholesterol (RemL-C) was measured using a homogenous assay (MetaboRead™, Kyowa Medex). In our previous studies, the serum concentration of RLP-C was measured using the immunoaffinity separation method[16]; however, the RLP-C concentration measured using this method is sometimes less than the lower limit of detection in young Japanese women[14]. In the present study, the serum concentration of RemL-C was therefore measured using a newly developed homogenous assay[16], and no samples were found to contain a concentration below the detection limit. The lipoprotein(a) (Lp(a)) level was measured using a latex agglutination method (Sekisui Medical). The levels of apolipoproteins (apo) AI, AII, CII, CIII and E were measured using an immunoturbidimetric method (Sekisui Medical). The level of apoB48 was measured using a chemiluminescent enzyme immunoassay (Fujirebio). The apoE phenotype

was measured using the isometric electrophoresis method (Phenotyping ApoE IEF System™, Joko, Tokyo, Japan).

Quantification of Postprandial Metabolism

Postprandial metabolism was quantified by calculating the incremental area under the curve (*Δ*AUC), which was defined as the difference between the area under the curve and the area below the baseline (0 hours) concentration from 0 to 6 hours, as previously described[14]. Postprandial changes in the concentrations of TG, RLP-TG, RemL-C, apoB and apoB48 were calculated as the difference from the baseline mean value (as 0 at 0 hours), and shown as *Δ*TG, *Δ*RLP-TG, *Δ*RemL-C, *Δ*apoB and *Δ*apoB48, respectively.

Statistics

All data are expressed as the mean ± SEM. The statistical analyses were performed using the SPSS ver. 19 software program (IBM, Tokyo, Japan). Differences in the time course compared with the fasting values were analyzed using the Friedman test followed by the Wilcoxon signed-rank test with the Bonferroni correction. The measured differences in the values at each time point in the four trials were assessed using the Kruskal-Wallis test followed by the Mann-Whitney U test with the Bonferroni correction.

Results

The physical characteristics and fasting blood chemical data of the subjects are shown in **Table 1**. There were no significant differences in any of the physical characteristics between the four trials.

Fructose, Glucose, Insulin, FFA and Lactate

The serum concentrations of fasting and postprandial fructose, glucose, insulin, FFA and lactate in the four trials are presented in **Table 2**, and the time courses of postprandial fructose, glucose, insulin, FFA and lactate are shown in **Fig.1A-E.**

The serum fructose concentrations in the Fru and Fru+Fat trials were significantly increased at 0.5, 1 and 2 hours compared with each fasting value ($p<0.05$). The concentration of fructose in the Fru trial was significantly higher than that observed in the Fru+Fat trial at 0.5 hours ($p<0.05$). At 2 hours, the fructose concentration was still significantly higher in the Fru and Fru+Fat trials compared

with that observed in the Glu and Fat trials (p<0.05 each). The concentration of fructose in the Fru and Fru+Fat trials fell to the fasting level at 4 hours.

The serum concentration of glucose in the Glu trial was significantly higher than that observed in the other three trials at 0.5 hours (p<0.05); however, it returned to the baseline value at 1 hour. Thereafter, there were no significant differences in the serum glucose concentration among the four trials.

The serum insulin concentration in the Glu trial was significantly higher than that observed in the other three trials at 0.5 hours (p<0.05). However, at 2 hours, there were no significant differences in the serum insulin concentration among the four trials, and no differences from the baseline values were observed.

The serum concentrations of FFA instantly decreased in the Glu, Fru, and Fru+Fat trials, reaching a minimum at 1 hour. The FFA concentration in the Fru and Fru+Fat trials was higher than that observed in the Glu trial at 1 (Fru vs. Glu p=0.19, Fru+Fat vs. Glu p=0.06) and 2 hours (Fru vs. Glu p=0.09, Fru+Fat vs. Glu p<0.05). However, the FFA concentration in the Fat trial did not decrease after the fat load and was higher than that observed in the other three trials at 1 and 2 hours (p<0.05 each).

The serum lactate concentration in the Fru and Fru+Fat trials significantly increased at 1 hour compared with each fasting value (p<0.05). At 1 hour, the serum concentration of lactate in the Fru trial was higher than that observed in the Fat trial (p<0.05) and the lactate concentration in the Fru+Fat trial was higher than that observed in the Glu and Fat trials (p<0.05). However, there were no significant differences in the serum lactate concentration among the four trials thereafter.

TG, RLP-TG and RemL-C

The concentrations of fasting and postprandial TG, RLP-TG and RemL-C in the four trials are presented in **Table 2**, and the time courses of ΔTG, ΔRLP-TG and ΔRemL-C are shown in **Table 3** and **Fig. 2A-C.** ΔTG, ΔRLP-TG and ΔRemL-C were calculated because the baseline value of serum TG in the Glu trial was significantly lower than that in the Fru trial (p<0.05). ΔTG peaked at 2 hours in the Fat trial and at 4 hours in the Fru+Fat trial. The ΔTG in the Fru+Fat trial was higher than that observed in the Fat trial at 4 and 6 hours (p<0.05). The ΔTG returned to the baseline value at 4 hours in the Fat trial and at 6 hours in the Fru+Fat trial. The ΔAUC-TG in the Fru+Fat trial was larger than that observed in the Fat trial (p<0.05) (**Table 4**).

The ΔRLP-TG peaked at 2 hours in the Fat trial and at 4 hours in the Fru+Fat

trial. The ⊿RLP-TG in the Fru+Fat trial was higher than that observed in the Fat trial at 4 hours (p<0.05). At 6 hours, the ⊿RLP-TG in the Fat trial, but not that in the Fru+Fat trial, returned to the baseline value. The ⊿AUC-RLP-TG in the Fru+Fat trial had a tendency to be larger than that observed in the Fat trial (p=0.15) (**Table 4**). The ⊿RemL-C in the Fat trial peaked at 2 hours, then decreased and returned to the baseline value at 4 hours. In contrast, the ⊿RemL-C in the Fru+Fat trial peaked at 4 hours and returned to the baseline value at 6 hours. The ⊿RemL-C in the Fru+Fat trial was higher than that observed in the Fat trial at 4 hours (p<0.05). The ⊿AUC-RemL-C in the Fru+Fat trial tended to be larger than that observed in the Fat trial (p=0.09) (**Table 4**).

apoB and apoB48

The concentrations of fasting and postprandial apoB and apoB48 in the four trials are presented in **Table 2**, with the time courses of ⊿apoB and ⊿apoB48 shown in **Table 3** and **Fig. 1D & E.** The ⊿apoB in the Fat, Fru+Fat and Glu trials was not significantly different than the fasting values observed over 6 hours. In contrast, the ⊿apoB in the Fru trial increased after 6 hours (p<0.05). The ⊿AUC-apoB did not differ among the four trials (**Table 4**).

The ⊿apoB48 in the Fat trial tended to increase at 1 hour (p=0.11) and peaked at 2 hours. However, the ⊿apoB48 in the Fru+Fat trial increased at 2 hours and peaked at 4 hours (p<0.05). The ⊿apoB48 in the Fru+Fat trial was significantly higher than that observed in the Fat trial at 4 and 6 hours (p<0.05). The ⊿apoB48 in the Fat trial, but not that in the Fru+Fat trial, returned to the baseline value at 6 hours. The ⊿AUC-apoB48 in the Fru+Fat trial was significantly larger than that observed in the Fat trial (p<0.05) (**Table 4**). The ⊿apoB48 in the Glu trial was slightly but significantly increased at 1 and 2 hours, then slightly decreased, then fell significantly below the baseline value at 6 hours. In contrast, the ⊿apoB48 in the Fru trial was not significantly different from the fasting value observed during the trial.

Discussion

The major finding of this study is that simultaneous ingestion of fructose and fat markedly enhanced postprandial lipidemia in young healthy Japanese women. In this study, the subjects ingested a moderate amount of fructose (0.5 g/kg body weight). Although other studies have employed 0.75-1.5 g/kg of fructose[7, 17], these

high amounts of fructose often induce malabsorption that causes gastrointestinal symptoms, for example bloating, abdominal pain, and diarrhea[18-20]. HFCS-sweetened beverages contain approximately 20-30 g/500 mL of fructose[21]. Therefore, we utilized a moderate amount of fructose (0.5 g/kg) that could be ingested daily. The daily fat intake in Japanese adult women is approximately 50 g[10]. Therefore, the amount of fat loaded in this study (0.35 g/kg) corresponds to approximately 1/3 of the daily fat consumption[13]. We demonstrated in a previous study that the postprandial lipoprotein metabolism can be evaluated in subjects who consume when this amount of fat[12-14]. In daily dietary habits, fructose is usually consumed with fat. For example, some beverages provided by fast-food restaurants, such as milk shakes, contain fat and HFCS. Therefore, we studied the effects of simultaneous ingestion of fructose and fat on postprandial lipoprotein and carbohydrate metabolism.

In the present study, the ingestion of fructose combined with fat led to a significantly higher rise and delayed peak in the serum TG, RLP-TG, and RemL-C concentrations compared with the ingestion of fat only. Insulin increases the expression and activity of lipoprotein lipase (LPL), and subjects who consume fructose have lower postprandial LPL activity than subjects who consume glucose[22]. The lower insulin excursion observed following fructose compared with glucose ingestion may result in a reduced activation of LPL, thus leading to a delayed TG clearance[7].

An interesting finding of this study is the change in the concentration of apoB48 following the simultaneous ingestion of fructose and fat; the concentration of apoB48 significantly increased after 2 hours, and the peak was delayed compared with that observed following the ingestion of fat only, suggesting delayed intestinal absorption of dietary fat or secretion of CM from the intestine in addition to late clearance of intestinal TRL, namely CM and chylomicron-remnant (CM-R). The concentration of apoB48 observed following glucose intake was slightly but significantly increased at 1 hour, then declined steadily, falling below the baseline at 6 hours, consistent with our previous results[23]. In contrast, the concentration of apoB48 did not change following fructose intake during the trial. These results suggest that the ingestion of glucose, but not fructose, may transiently stimulate the synthesis and/or secretion of intestinal apoB48-containing lipoproteins.

Although postprandial dyslipidemia was previously regarded to be a consequence of delayed TRL clearance, emerging implicates intestinal overproduction of apoB48-containing lipoproteins as a major contributor to postprandial

dyslipidemia[24]. In the present study, the concentration of apoB48 markedly increased and its peak was delayed following intake of fructose combined with fat compared with that observed following intake of fat only. In addition, the concentration of apoB48 was lower and its peak was earlier (at 2 hours) following intake of glucose combined with fat in our previous study[23] compared with that observed following intake of fructose combined with fat in the present study. These results suggest the retention of CM-R in the circulation following ingestion of fructose combined with fat compared with that observed following ingestion of glucose with fat. Delayed CM secretion from the intestine depends on differences in the absorption mechanisms of fructose and glucose. Fructose is transported into enterocytes through a specific fructose transporter, GLUT-5, located in the intestinal membrane. The absorption of fructose via facilitated diffusion, which does not require adenosine 5'-triphosphate, differs from that observed in the glucose absorption system[25, 26]. Therefore, the simultaneous ingestion of fructose and fat may have delayed the absorption of fat and the secretion of apoB48-containing TRL particles.

Fructose ingestion is known to acutely suppress plasma FFA and whole-body lipid oxidation[27]. The integrated postprandial inhibition of plasma FFA is of comparable magnitude following the ingestion of equivalent amounts of glucose and fructose, as shown in the present study. The slight increase in plasma insulin elicited by fructose may be sufficient to explain the inhibition of adipose tissue lipolysis, although this increase is quite modest compared with that observed following glucose ingestion.

In addition, the concentration of lactate increased following the ingestion of fructose (but not glucose) or fructose combined with fat. The increased lactate production most likely occurred because the fructokinase activity increased, the rate-limiting step for glycolysis (phosphofructokinase) was bypassed, and the pyruvate kinase activity was stimulated by the accumulation of fructose-1-phosphate[28]. However, part of the fructose absorbed into enterocytes may be converted into lactate and released into the portal circulation[1]. In vitro data indicate that lactate rather than triose phosphate is the primary lipogenic precursor stimulated following fructose administration and that activation of pyruvate dehydrogenase by a high-fructose diet is a major regulatory step in this process[29]. Simultaneously, fructose inhibits hepatic lipid oxidation, thereby favoring fatty acid reesterification and VLDL-TG synthesis[30]. Triose phosphate produced from fructose is subsequently converted into pyruvate and oxidized into CO_2 and H_2O in the

tricarboxylic acid cycle. A portion of the triose phosphate produced is converted into lactate, accounting for the increase in plasma lactate concentrations observed following fructose ingestion in the present study[31].

Fructose has the same chemical formula as glucose, namely $C_6H_{12}O_6$; however, its metabolism differs markedly from that of glucose due to its almost complete hepatic extraction and rapid hepatic conversion into glucose, glycogen, lactate, and fat. A chronically high fructose intake has been shown to cause dyslipidemia and impair hepatic insulin sensitivity[1]. A meta-analysis concluded that a fructose intake of >50 g/day (i.e., close to the average daily intake in the USA) is associated with increased postprandial TG excursions, while a fructose intake of >100 g/day is associated with increased fasting TG levels[32]. Diets containing >15% of energy derived from fructose, compared with glucose are consistently associated with increases in both fasting and postprandial TG concentrations in humans[33-36]. Chronic consumption of fructose increases the deposition of visceral adipose tissue, while the consumption of glucose results primarily in an increased deposition of subcutaneous adipose tissue[22]. The chronic consumption of fructose is also associated with an increased concentration of small, dense LDL particles[37]. The adverse effects of fructose leading may be explained by its hepatic metabolism which is independent from the energy status, leading to unregulated hepatic fructose uptake and increased lipogenesis[22]. As shown in the present study and a study conducted by Chong et al[7], after the ingestion of fructose, the peak plasma fructose concentration remains at approximately 10 mg/dL, thus indicating that first-pass hepatic extraction is much higher than that observed in the glucose ingestion. In the liver, fructose is metabolized into glyceraldehyde and dihydroxyacetone phosphate. The ability of fructose to bypass the primary regulatory step of glycolysis, the conversion of glucose-6-phosphate to fructose 1,6-bisphosphate controlled by phosphofructokinase, is of key importance. Therefore, while the glucose metabolism is negatively regulated by phosphofructokinase, fructose can continuously enter the glycolytic pathway in an unregulated manner[38]. In the present study, the concentration of apoB(100) was increased in the trial of ingestion of fructose only, and not in other three trials, thus suggesting that the ingestion of fructose may increase VLDL-apoB100 production in the liver.

Sex differences in fasting plasma TG concentrations are generally attributed to lower VLDL-TG concentration in women. Increased clearance of VLDL-TG in plasma is responsible for the lower VLDL-TG concentration, whereas a deceased VLDL-apoB100 secretion rate, combined with a shorter residence time of

VLDL-apoB100 in plasma, is responsible for the lower VLDL-apoB100 concentrations observed in women[39]. In women, the liver secretes VLDL particles that contain more TG, and are therefore larger, than the VLDL particles secreted by the liver in men. The results of in vivo studies demonstrate faster removal of TG from large, TG-rich VLDL particles than from small, TG-poor particles[40]. The present study demonstrated that the simultaneous ingestion of a moderate amount of fructose and fat, which can be ingested daily, markedly exacerbates postprandial exogenous lipidemia, even in young healthy women. Although postprandial lipidemia is a known risk factor for coronary heart disease[8], the influence of high fructose intake on health in young women has not been clarified. We are apprehensive of the deleterious effects of fructose ingestion on the health of this population. However, due to the relatively small number of subjects enrolled, the present results should be interpreted with caution.

Conclusion

Our present results demonstrated that the simultaneous ingestion of a moderate amount of fructose and fat markedly exacerbates postprandial exogenous lipidemia in young healthy Japanese women, thus suggesting that even a relatively modest amount of fructose may be excessive.

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Table 1. Anthropometric and clinical characteristics.

Trial	Fru	Fat	Fru+Fat	Glu
Age (years)	21.2 ± 0.3			
Height (cm)	160.4 ± 1.9			
Weight (kg)	52.8 ± 2.3	52.8 ± 2.4	52.7 ± 2.3	53.1 ± 2.4
BMI (kg/m^2)	20.5 ± 0.7	20.5 ± 0.8	20.5 ± 0.7	20.6 ± 0.8
Waist (cm)	68.9 ± 1.6	70.2 ± 1.7	69.1 ± 1.6	71.1 ± 1.6
W/H	0.75 ± 0.01	0.78 ± 0.02	0.76 ± 0.01	0.77 ± 0.01
VFA (cm^2)	24.5 ± 4.3	26.6 ± 3.9	26.3 ± 3.8	28.0 ± 4.8
HbA1c (NGSP) (%)	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1
HOMA-IR	1.5 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	1.4 ± 0.1
TC (mg/dL)	176.6 ± 6.9	181.9 ± 6.3	182.3 ± 9.0	179.8 ± 11.1
LDL-C (mg/dL)	99.2 ± 7.3	103.8 ± 6.7	104.6 ± 8.4	104.9 ± 9.6
sdLDL-C (mg/dL)	23.3 ± 1.8	22.3 ± 1.2	25.1 ± 2.4	24.2 ± 2.7
HDL-C (mg/dL)	63.6 ± 3.7	66.8 ± 3.6	64.6 ± 3.2	65.4 ± 4.2
Lp(a) (mg/dL)	7.6 ± 6.3			
apoAI (mg/dL)	154.8 ± 8.1	154.9 ± 7.0	154.9 ± 7.5	153.7 ± 10.8
apoAII (mg/dL)	27.1 ± 1.3	27.1 ± 0.9	28.2 ± 1.3	27.1 ± 1.4
apoCII (mg/dL)	2.7 ± 0.2	2.5 ± 0.2	2.8 ± 0.3	2.3 ± 0.2
apoCIII (mg/dL)	7.8 ± 0.6	7.7 ± 0.5	8.1 ± 0.7	7.3 ± 0.7
apoE (mg/dL)	3.8 ± 0.2	4.1 ± 0.3	4.0 ± 0.3	3.8 ± 0.3
SBP (mmHg)	99.1 ± 1.8	99.9 ± 2.5	97.7 ± 2.3	100.1 ± 2.4
DBP (mmHg)	61.9 ± 1.6	64.8 ± 2.2	63.0 ± 2.3	64.1 ± 3.1
Pulse rate (beats/min)	67.0 ± 2.3	68.0 ± 2.4	67.9 ± 2.4	65.6 ± 2.1

All values are presented as mean ± SEM.

SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: pulse rate

Table 2. The parameters before and after beverage ingestion.

	Time (h)	0	0.5	1	2	4	6
Fructose (mg/dL)	Fru	0.54 ± 0.08	11.44 ± 0.93*#	7.60 ± 0.70*#	2.64 ± 0.41*#	0.79 ± 0.12	0.56 ± 0.03
	Fat	0.56 ± 0.07	0.77 ± 0.10#	0.68 ± 0.07#	0.77 ± 0.08#	0.71 ± 0.08	0.42 ± 0.06
	Fru+Fat	0.60 ± 0.06	6.68 ± 0.44*#	7.30 ± 0.48*#	3.34 ± 0.45*#	1.02 ± 0.08	0.55 ± 0.07
	Glu	0.52 ± 0.09	0.64 ± 0.06#	0.70 ± 0.06#	0.79 ± 0.09#	0.78 ± 0.05	0.58 ± 0.10
Glucose (mg/dL)	Fru	95.6 ± 1.6	101.2 ± 3.1#	96.5 ± 2.2	92.8 ± 2.9	91.5 ± 2.2	89.9 ± 2.9
	Fat	93.1 ± 2.8	89.9 ± 2.6#	91.8 ± 2.8#	91.5 ± 1.5	90.1 ± 1.2	84.1 ± 1.0
	Fru+Fat	94.9 ± 2.0	99.8 ± 2.6#	97.7 ± 3.1#	91.7 ± 2.6	93.5 ± 2.0	90.4 ± 1.3
	Glu	95.6 ± 2.4	133.5 ± 6.0*#	128.5 ± 8.4#	87.0 ± 3.2	88.1 ± 2.1*	88.6 ± 2.2
Insulin (μU/mL)	Fru	6.42 ± 0.64	14.69 ± 1.67*#	9.77 ± 0.94*#	6.58 ± 0.70	3.98 ± 0.35	3.55 ± 0.23*
	Fat	5.78 ± 0.80	10.08 ± 1.29#	8.67 ± 1.02*#	6.63 ± 0.87	3.85 ± 0.49	2.99 ± 0.27*
	Fru+Fat	6.57 ± 0.94	16.20 ± 2.60*#	12.72 ± 2.03*#	8.45 ± 1.04	4.80 ± 0.92	4.09 ± 0.71*
	Glu	6.00 ± 0.33	42.39 ± 4.98*#	28.00 ± 4.69*#	7.50 ± 1.23	3.62 ± 0.42*	3.67 ± 0.33*
FFA (μmol/L)	Fru	499.2 ± 47.6	167.3 ± 27.5*#	227.8 ± 23.4*#	557.2 ± 46.4	682.0 ± 79.1	
	Fat	486.8 ± 61.2	448.2 ± 42.6#	573.8 ± 36.2#	711.8 ± 51.9	816.7 ± 62.2*	
	Fru+Fat	416.2 ± 40.3	198.9 ± 20.2*#	253.7 ± 34.9*#	512.0 ± 61.4	626.8 ± 55.8*	
	Glu	440.9 ± 39.6	102.0 ± 25.7*#	120.8 ± 28.3*#	599.1 ± 64.6	788.8 ± 40.4*	
Lactate (mg/dL)	Fru	9.9 ± 1.0	18.6 ± 1.8*#	10.8 ± 1.4	8.4 ± 0.9	9.4 ± 1.1	
	Fat	12.0 ± 1.5	8.4 ± 1.3*#	9.9 ± 1.3	9.8 ± 1.2	9.7 ± 1.0	
	Fru+Fat	12.0 ± 1.7	21.2 ± 2.3*#	14.7 ± 1.7	9.5 ± 1.0	9.2 ± 1.1	
	Glu	11.3 ± 1.3	11.9 ± 0.9#	9.6 ± 1.2	8.0 ± 0.8	9.7 ± 0.9	
TG (mg/dL)	Fru	69.2 ± 8.1#	64.8 ± 7.3	61.8 ± 6.4*	74.8 ± 7.1	77.3 ± 7.1#	
	Fat	56.4 ± 5.2#	63.4 ± 6.1	74.9 ± 8.4*#	62.2 ± 6.3#	48.9 ± 4.4	
	Fru+Fat	66.0 ± 6.5#	69.4 ± 7.3#	88.0 ± 12.0*#	129.4 ± 24.1*#	98.4 ± 24.9	
	Glu	47.2 ± 3.4#	46.6 ± 3.0#	42.6 ± 3.0#	53.7 ± 4.0#	54.8 ± 4.8	
RLP-TG (mg/dL)	Fru	10.4 ± 1.3	8.8 ± 0.9	8.5 ± 0.9#	10.6 ± 1.3#	10.1 ± 1.2#	
	Fat	8.3 ± 0.9	13.3 ± 2.5	19.8 ± 2.8*#	14.2 ± 1.9*#	10.3 ± 1.2	
	Fru+Fat	10.7 ± 2.2	12.6 ± 2.2*	24.8 ± 5.1*#	44.0 ± 15.1*#	26.4 ± 13.0*#	
	Glu	7.4 ± 0.6	8.4 ± 0.3	6.5 ± 0.3#	7.7 ± 0.4#	8.6 ± 0.8	
RemL-C (mg/dL)	Fru	6.1 ± 1.0	5.6 ± 0.8	5.4 ± 0.7*	6.4 ± 0.8	6.9 ± 0.9*	
	Fat	5.0 ± 0.4	5.4 ± 0.5	6.2 ± 0.7*#	5.8 ± 0.6#	5.0 ± 0.5	
	Fru+Fat	6.1 ± 0.8	6.1 ± 0.8	7.1 ± 1.1#	10.5 ± 1.7*#	9.5 ± 2.3	
	Glu	4.4 ± 0.4	4.2 ± 0.4	3.8 ± 0.4#	4.7 ± 0.4#	5.0 ± 0.5	
apoB (mg/dL)	Fru	74.1 ± 4.4	74.1 ± 5.0	74.2 ± 5.0	75.0 ± 4.7	76.1 ± 4.7*	
	Fat	74.8 ± 4.5	73.4 ± 4.4	73.4 ± 4.7	74.2 ± 4.5	75.3 ± 4.6	
	Fru+Fat	77.4 ± 4.9	75.7 ± 4.7	74.9 ± 4.7	76.8 ± 4.7	77.8 ± 4.8	
	Glu	75.4 ± 5.6	73.0 ± 6.0	74.2 ± 5.9	75.8 ± 6.0	75.7 ± 6.0	
apoB48 (μg/mL)	Fru	2.3 ± 0.3	1.9 ± 0.3	2.0 ± 0.3#	2.5 ± 0.3#	2.2 ± 0.3#	
	Fat	1.9 ± 0.2	3.1 ± 0.4	3.9 ± 0.4*#	3.2 ± 0.4*#	2.9 ± 0.3#	
	Fru+Fat	2.6 ± 0.4	2.8 ± 0.5	4.8 ± 0.5*#	6.8 ± 0.8*#	5.0 ± 1.0*#	
	Glu	1.9 ± 0.2	2.8 ± 0.4*	2.6 ± 0.3*#	1.5 ± 0.2#	1.1 ± 0.2*#	

All values are presented as mean ± SEM. *p<0.05 compared to the fasting values. #p<0.05 compared between the trials.

Table 3. The parameters before and after beverage ingestion.

	Time (h)	0	1	2	4	6
⊿TG (mg/dL)	Fru	0.0 ± 0.0	-4.4 ± 2.4	-7.4 ± 2.7*	5.6 ± 1.8	8.1 ± 2.4
	Fat	0.0 ± 0.0	7.0 ± 3.0	18.4 ± 3.9*	5.8 ± 3.3	-7.6 ± 4.1
	Fru+Fat	0.0 ± 0.0	3.4 ± 2.4	22.0 ± 6.4*	63.4 ± 17.8*	32.4 ± 18.7
	Glu	0.0 ± 0.0	-0.7 ± 1.4	-4.7 ± 2.0	6.4 ± 2.7	7.6 ± 3.1
⊿RLP-TG (mg/dL)	Fru	0.0 ± 0.0	-1.6 ± 0.6	-1.9 ± 0.8	0.2 ± 0.9	-0.3 ± 0.7
	Fat	0.0 ± 0.0	4.9 ± 2.3	11.5 ± 2.1*	5.8 ± 1.4*	1.9 ± 1.1
	Fru+Fat	0.0 ± 0.0	2.0 ± 0.7*	14.1 ± 3.4*	33.4 ± 13.1*	15.7 ± 10.9*
	Glu	0.0 ± 0.0	1.0 ± 0.7	-1.0 ± 0.6	0.2 ± 0.8	1.2 ± 0.9
⊿RemL-C (mg/dL)	Fru	0.0 ± 0.0	-0.5 ± 0.2	-0.8 ± 0.3*	0.4 ± 0.2	0.8 ± 0.2*
	Fat	0.0 ± 0.0	0.4 ± 0.2	1.1 ± 0.3*	0.8 ± 0.3	0.0 ± 0.5
	Fru+Fat	0.0 ± 0.0	0.0 ± 0.2	1.0 ± 0.4	4.3 ± 1.0*	3.4 ± 1.6
	Glu	0.0 ± 0.0	-0.1 ± 0.1	-0.5 ± 0.1	0.3 ± 0.2	0.6 ± 0.3
⊿apoB (mg/dL)	Fru	0.0 ± 0.0	0.0 ± 0.8	0.1 ± 0.8	0.9 ± 0.9	2.0 ± 0.5*
	Fat	0.0 ± 0.0	-1.3 ± 0.4	-1.3 ± 0.6	-0.6 ± 0.7	0.6 ± 0.8
	Fru+Fat	0.0 ± 0.0	-1.8 ± 0.9	-2.6 ± 1.1	-0.7 ± 1.3	0.3 ± 1.5
	Glu	0.0 ± 0.0	-2.4 ± 0.9	-1.2 ± 0.9	0.3 ± 1.1	0.2 ± 1.0
⊿apoB48 (μg/mL)	Fru	0.0 ± 0.0	-0.5 ± 0.1	-0.3 ± 0.2	0.2 ± 0.2	-0.1 ± 0.2
	Fat	0.0 ± 0.0	1.2 ± 0.4	2.0 ± 0.3*	1.3 ± 0.3*	1.0 ± 0.4
	Fru+Fat	0.0 ± 0.0	0.2 ± 0.2	2.2 ± 0.3*	4.2 ± 0.4*	2.4 ± 0.7*
	Glu	0.0 ± 0.0	0.9 ± 0.2*	0.7 ± 0.2*	-0.4 ± 0.1	-0.8 ± 0.1*

All values are presented as mean ± SEM. *p<0.05 compared to the fasting values. #p<0.05 compared between the trials.

Table 4. The incremental area under the curve (⊿AUC) for TG, RLP-TG, RemL-C, apoB and apoB48.

Trial	⊿AUC-TG (mg･h/dL)	⊿AUC-RLP-TG (mg･h/dL)	⊿AUC-RemL-C (mg･h/dL)	⊿AUC-apoB (mg･h/dL)	⊿AUC-apoB48 (μg･h/mL)
Fru	3.6 ± 11.2	-4.2 ± 3.6	-0.2 ± 1.1	3.9 ± 3.8	-0.7 ± 0.7
Fat	38.7 ± 10.4	35.8 ± 5.1	3.8 ± 1.4	-3.9 ± 3.1	7.9 ± 1.3
Fru+Fat	195.8 ± 62.9	105.6 ± 40.7	13.5 ± 4.1	-6.6 ± 6.3	14.4 ± 1.4
Glu	12.8 ± 12.0	1.2 ± 3.6	0.4 ± 1.0	-3.4 ± 5.1	0.5 ± 0.7

All values are presented as mean ± SEM. $*p<0.05$

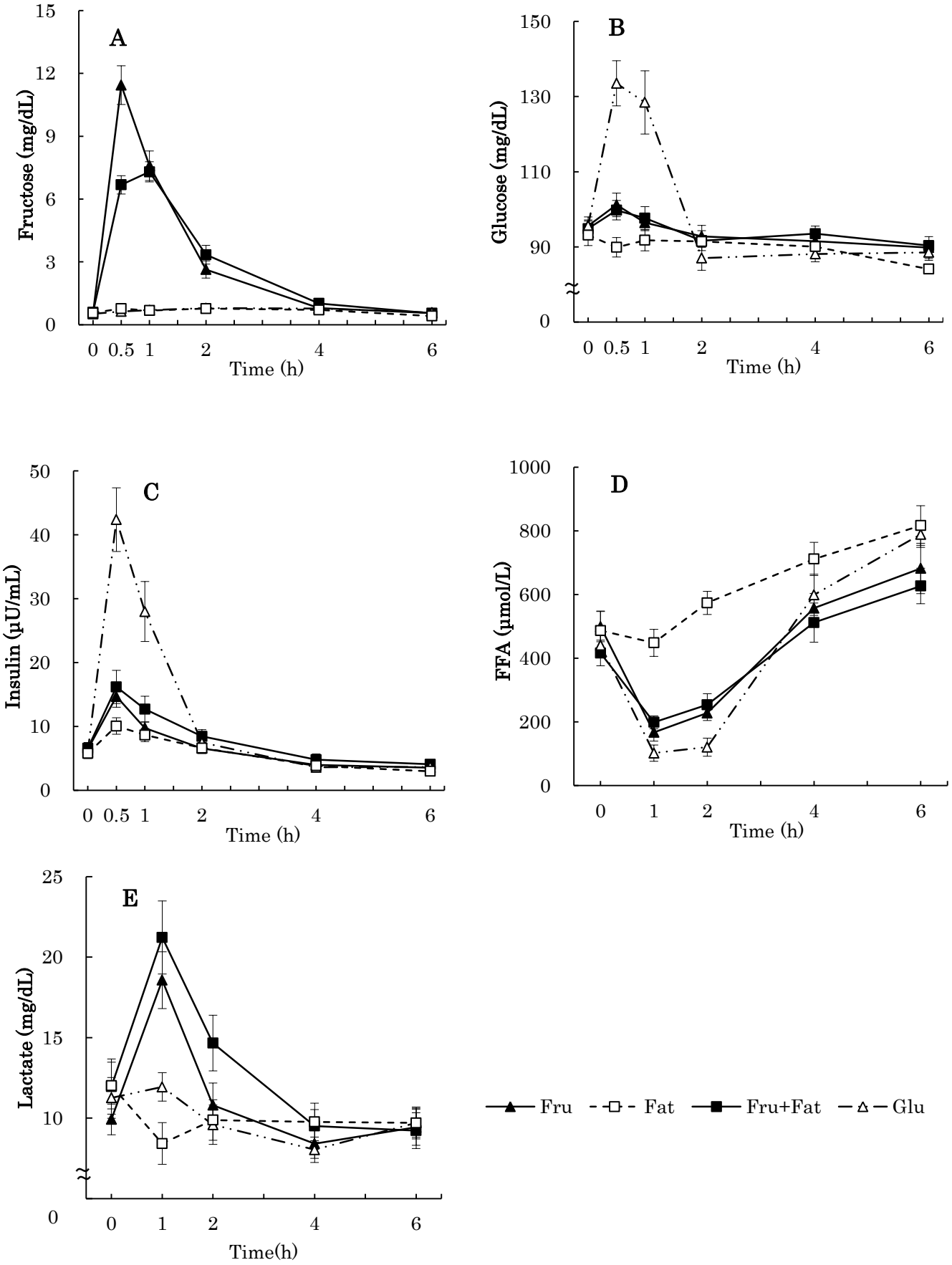

Fig. 1. Postprandial Fructose (A), Glucose (B), Insulin (C), FFA (D) and Lactate (E). The values are presented as mean ±SEM.

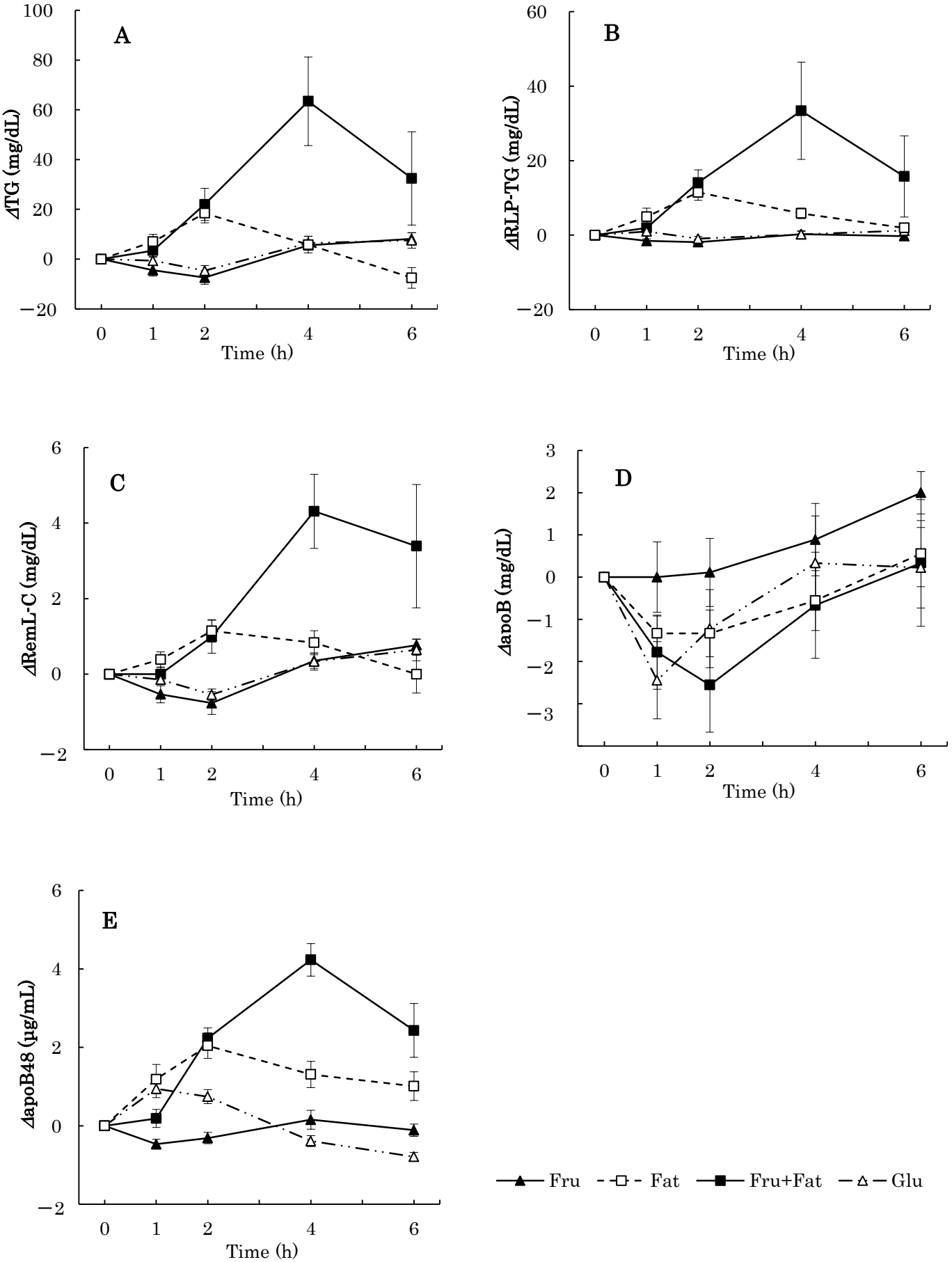

Fig. 2. Postprandial ⊿TG (A), ⊿RLP-TG (B), ⊿RemL-C (C), ⊿apoB (D) and ⊿apoB48 (E). The values are presented as mean ±SEM.

Chapter 2

The ingestion of a fructose-containing beverage combined with fat cream exacerbates postprandial endogenous lipidemia in young healthy women (Manuscript submitted to Journal of Atherosclerosis and Thrombosis for publication)

Abstract

Aim: To investigate the acute effects of the ingestion of a fructose-containing beverage, particularly when combined with fat, on postprandial lipoprotein metabolism.

Methods: Twelve young healthy Japanese women with apolipoprotein E phenotype 3/3 were enrolled in the study. At each of four sessions, the subjects ingested one of four sugar beverages containing fructose and/or glucose (totally 0.5 g/kg body weight) combined with OFTT cream (1 g/kg, 0.35 g/kg as fat) in a randomized crossover design. The four sugar beverages were: 100% (w/w) fructose (F100), 90% fructose+10% glucose (F90G10), 55% fructose+45% glucose (F55G45), and 100% glucose (G100). Venous blood samples were taken at baseline and at 0.5, 1, 2, 4 and 6 hours after ingestion.

Results: The concentration at 4 hours and the incremental area under the curve (ΔAUC) of hepatic triglyceride-rich lipoprotein-triglyceride in the F100 and F90G10 trials were significantly higher or larger, respectively, than those observed in the G100 trial. However, the concentration of apolipoprotein B(-100) did not change during the 6 hours. The concentration of apolipoprotein B-48 peaked at 2 hours in the G100 trial, but at 4 hours in the other trials, and did not return to baseline at 6 hours except the G100 trial. At 4 hours, the concentration of apolipoprotein B-48 in the F100 and F90G10 trials tended to be higher than that in the G100 trial.

Conclusion: The ingestion of a high-fructose-containing beverage with fat cream delayed the clearance of chylomicron and its remnant derived from the intestine, and enhanced the secretion of triglyceride-rich lipoprotein particles from the liver, inducing postprandial lipidemia, even in young healthy women.

Introduction

The consumption of high-fructose corn syrup (HFCS) as an alternative to sucrose has been increasing in Japan since 1980. Soft drinks account for approximately 50% of the total consumption of HFCS[1]. HFCS is classified into three different types: high-fructose liquid sugar (fructose≥90%), fructose-glucose liquid sugar (50%≤fructose<90%), and glucose-fructose liquid sugar (fructose<50%)[2]. Of these, the most commonly used is fructose-glucose liquid sugar[3].

Overconsumption of fructose can have adverse effects on human health. Diets containing >15% of energy derived from fructose, as compared with glucose, are consistently associated with increases in both fasting and postprandial triglyceride (TG) concentrations[4, 5], and postprandial hyperlipidemia is widely accepted to be a major risk factor for coronary heart disease, as was first proposed by Zilversmit[6]. Habitual consumption of fructose has also been reported to increase the deposition of visceral adipose tissue[7]. In our previous study, serum concentrations of TG, remnant-like particle (RLP)-TG, and apolipoprotein B-48 (apoB48) were significantly increased following the ingestion of moderate amount of fructose (0.5 g/kg body weight) combined with fat, compared to ingestion of glucose, in healthy young women even in a single ingestion, demonstrating that fructose markedly exacerbates postprandial exogenous lipidemia. In that study, however, we did not investigate the endogenous TG-rich lipoprotein (TRL) metabolism in detail[8].

In our previous study, subjects ingested a beverage containing fructose (without glucose) combined with fat cream. In reality, however, fructose is usually consumed as HFCS in soft drinks and many other foods[9]. In the present study, we enrolled young healthy women, thought to be among the highest consumers of fructose, and investigated the TRL changes derived from the intestine and the liver following the ingestion of fructose and/or glucose combined with fat cream.

Aim

To elucidate the acute effects of the ingestion of a fructose-containing beverage combined with fat cream on postprandial exogenous and endogenous lipoprotein metabolism in young healthy Japanese women.

Methods

Subjects

Twelve young healthy Japanese women with normal ovarian cycles and apolipoprotein E (apoE) phenotype 3/3 were enrolled as participants. All subjects were non-smokers, had no apparent acute or chronic illnesses, and were not taking any medications or dietary supplements. This study was approved by the Institutional Review Board of the Sugiyama Jogakuen University School of Life Studies, and each subject gave written informed consent for study participation.

Anthropometric and body composition measurement

Body weight and height were measured according to standard methods. Waist circumference was assessed as the abdominal girth at the level of the umbilicus, and hip circumference was measured at the level of the greater trochanters. The waist-to-hip (W/H) ratio was calculated. Body composition, including the visceral fat area (VFA), was analyzed using an eight-polar bioelectrical impedance method with the InBody720 (Biospace, Tokyo, Japan).

Sugar and fat load test

Each subject underwent four test trials. At each test trial, the subjects ingested one of four beverages containing fructose (F) and/or glucose (G) (totally 0.5 g/kg body weight) combined with oral fat tolerance test (OFTT) cream (Jomo, Takasaki, Japan; 1 g/kg, 0.35 g/kg as fat) in a randomized crossover design. OFTT cream was used as previously described[10-13]. The four beverages were prepared as follows; F100: 100% (w/w) fructose (Nisshin Seito, Tokyo, Japan), F90G10: 90% fructose+10% glucose (Fuso Pharmaceutical, Osaka, Japan), F55G45: 55% fructose+45% glucose, and G100: 100% glucose.

Experimental design

Subjects abstained from consuming caffeine or alcohol on the day before the experiment and ingested one of the four beverages after a 12-hour overnight fast. Venous blood samples were obtained before (0 hours) and at 0.5, 1, 2, 4 and 6 hours after ingestion. During the test, subjects avoided exercise and eating, but had free access to water after 1 hour. All blood samples were obtained while subjects were in a supine position. There was an interval of four weeks between the test days to minimize the confounding effects of the subject's menstrual status on lipid metabolism.

Biochemical analysis

The serum samples were immediately refrigerated at 4°C or frozen at −80°C until analysis. The level of fructose was measured enzymatically (BioAssay Systems, CA, USA). The level of glucose was measured using a mutarotase GOD method (Wako, Osaka, Japan). The level of insulin was measured using a chemiluminescent enzyme immunoassay (Fujirebio, Tokyo, Japan). Insulin resistance was evaluated according to the homeostasis model assessment for insulin resistance (HOMA-IR)[14]. The hemoglobin A1c (HbA1c) level was measured using a latex agglutination method (Fujirebio) and expressed as the National Glycohemoglobin Standardization Program (NGSP) value. The levels of free fatty acids (FFA) (Eiken Chemical, Tokyo, Japan) and lactate (Kyowa Medex, Tokyo, Japan) were measured enzymatically. The level of β-hydroxybutyric acid (β-HB) was measured using an enzymatic cycling method (Kainos, Tokyo, Japan). The level of total cholesterol (TC) was measured enzymatically (Sysmex, Hyogo, Japan). The level of high-density lipoprotein-cholesterol (HDL-C) was measured using a direct method (Fujirebio), while the level of low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula. The level of TG was measured enzymatically (Sekisui Medical, Tokyo, Japan).

The TG level in the TG-rich lipoproteins was estimated using a newly developed homogenous assay (VLDL-TG™, Shino-test, Tokyo, Japan)[15] and shown as hepatic triglyceride-rich lipoprotein-triglyceride (hTRL-TG). hTRL-TG includes TG in endogenous lipoproteins (VLDL and its remnant), but not TG in exogenous lipoproteins (CM and its remnant). The level of RLP-TG was measured using an immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan), and the level of remnant lipoprotein-cholesterol (RemL-C) using a homogenous assay (MetaboRead™, Kyowa Medex, Japan). The level of lipoprotein(a) (Lp(a)) was measured using a latex agglutination method (Sekisui Medical). The level of apolipoproteins AI, AII, B(-100), CII, CIII and E were measured using an immunoturbidimetric method (Sekisui Medical). The level of apoB48 was measured by chemiluminescent enzyme immunoassay (Fujirebio). The apoE phenotype was measured using the isometric electrophoresis method (Phenotyping ApoE IEF System™, Joko, Tokyo, Japan).

Quantification of postprandial metabolism

Postprandial changes in the concentrations of TG, hTRL-TG, RLP-TG, RemL-C, apoB and apoB48 were calculated as the difference from the baseline mean value

(as 0 at 0 hours), and shown as ⊿TG, ⊿hTRL-TG, ⊿RLP-TG, ⊿RemL-C, ⊿apoB and ⊿apoB48, respectively. Postprandial metabolism was quantified by calculating the incremental area under the curve (⊿AUC), which was defined as the difference between the area under the curve and the area below the baseline (0 hours) concentration from 0 to 6 hours, as previously described[8].

Statistics

All data are expressed as mean ± SEM. The statistical analyses were performed using SPSS ver. 19 (IBM, Tokyo, Japan). Differences in the time course compared with the fasting values were analyzed using the Friedman test, followed by the Wilcoxon signed-rank test with the Bonferroni correction. The differences in the values at each time point in the four trials were assessed using Kruskal-Wallis test, followed by Mann-Whitney U-test with the Bonferroni correction.

Results

The physical characteristics and fasting blood chemistry data of the subjects are shown in **Table 1**. There were no significant differences in any of the physical characteristics among the four trials.

Fructose, glucose, insulin, FFA, β-HB and lactate

The serum concentrations of fasting and postprandial fructose, glucose, insulin, FFA, β-HB and lactate in the four trials are presented in **Table 2, Fig. 1.**

The serum fructose concentration in the F100, F90G10 and F55G45 trials was significantly higher than in the G100 trial at 0.5, 1 and 2 hours ($p<0.05$ each). At 4 and 6 hours, there were no significant differences in the serum fructose concentration among the four trials.

The serum concentration of glucose was significantly increased at 0.5 hours in the G100 and F90G10 trials compared with each fasting value ($p<0.05$ each). The glucose concentration in the G100 trial tended to be higher than that observed in the F100 ($p=0.06$) and F90G10 ($p=0.15$) trials at 0.5 hours. At 1 hour, there were no significant differences in the serum glucose concentration among the four trials.

The serum concentration of insulin in the F100 and F90G10 trials was lower than that observed in the G100 trial at 0.5 hours (F100 vs. G100 $p<0.05$, F90G10 vs. G100 $p=0.09$) and 1 hour (F100 and F90G10 vs. G100 $p<0.05$).

The serum concentration of FFA instantly decreased in the G100, F55G45, and

F90G10 trials, reaching its lowest level at 1 hour in these trials, but at 2 hours in the F100 trial, followed by an increase in all trials. At 4 and 6 hours, the serum FFA concentration in the F100 and F90G10 trials was lower, but not significantly, than that observed in the G100 trial.

The serum β-HB concentration at 4 and 6 hours in the four trials was significantly higher compared to each fasting value ($p<0.05$ each). At 4 and 6 hours, the serum β-HB concentration in the F100 and F90G10 trial was lower, but not significantly, than that observed in the G100 trial.

The serum lactate concentration in the F100 and F90G10 trials was increased at 1 hour ($p<0.05$ each) and 2 hours (F100 $p<0.05$, F90G10 $p=0.17$) compared to each fasting value, but returned to baseline at 4 hours. At 2 hours, the serum lactate concentration in the F100 ($p<0.05$) and F90G10 ($p=0.08$) trials was higher than that observed in the G100 trial.

TG, hTRL-TG, RLP-TG and RemL-C

The concentrations of fasting and postprandial TG, hTRL-TG, RLP-TG and RemL-C in the four trials are presented in **Table 3**, and the time courses of ⊿TG, ⊿hTRL-TG, ⊿RLP-TG and ⊿RemL-C are shown in **Table 4**, **Fig. 2A-D.**

The ⊿TG in the F100, F90G10 and F55G45 trials was significantly higher compared with each fasting level at 2 and 4 hours ($p<0.05$), and returned to the fasting level at 6 hours except in the case of the F100 trial. The ⊿TG in the G100 trial tended to be higher at 2 hours ($p=0.11$) compared to the fasting level. The ⊿AUC-TG in the F100 trial tended to be larger than in the G100 ($p=0.09$) and F55G45 ($p=0.13$) trials (**Table 5**, **Fig. 3A**).

The ⊿hTRL-TG at 2 hours in the F100 ($p=0.06$), F90G10 ($p<0.05$) and F55G45 ($p=0.10$) trials was higher than each fasting level, peaked at 4 hours, then returned to each fasting value at 6 hours except in the case of the F100 trial. The ⊿hTRL-TG in the G100 trial showed no significant change during the 6 hours. The ⊿hTRL-TG at 4 hours in the F100 and F90G10 trials was significantly higher than that in the G100 trial ($p<0.05$ each). In the F100 trial, the ⊿AUC-hTRL-TG was significantly larger than that observed in the G100 and F55G45 trials ($p<0.05$ each), while in the F90G10 trial, the ⊿AUC-hTRL-TG was significantly larger than that observed in the G100 trial ($p<0.05$) (**Table 5**, **Fig. 3B**).

The ⊿RLP-TG at 1 hour was significantly higher than each fasting value in the F55G45 and G100 trials ($p<0.05$ each), but at 2 hours in the F100 and F90G10 trials ($p<0.05$ each). The ⊿RLP-TG peaked at 2 hours in the G100 trial and at 4 hours in

the other trials. In all trials except the G100, the ⊿RLP-TG did not return to each fasting level at 6 hours although the ⊿AUC-RLP-TG had no significant differences among the four trials.

The ⊿RemL-C in all trials except the G100 trial was significantly increased at 4 and 6 hours (p<0.05 each). In the G100 trial, the ⊿RemL-C was not significantly different compared to the fasting value throughout the 6 hours. The ⊿RemL-C in the F100 and F90G10 trials was higher than in the G100 trial at 4 hours (F100 vs. G100 p<0.05, F90G10 vs. G100 p=0.15) and at 6 hours (F100 vs. G100 p<0.05, F90G10 vs. G100 p=0.06). The ⊿AUC-RemL-C in the F100 trial was larger than that observed in the G100 and F55G45 trials (p<0.05 each) (**Table 5**, **Fig. 3C**).

apoB and apoB48

The concentrations of fasting and postprandial apoB and apoB48 in the four trials are presented in **Table 3**, and time courses of ⊿apoB and ⊿apoB48 are shown in **Table 4**, **Fig. 2E & F.**

The ⊿apoB in the F90G10, F55G45 and G100 trials was significantly decreased at 1 and 2 hours, but only at 2 hours in the F100 trial (p<0.05 each). In all four trials, the ⊿apoB returned to the fasting values at 4 hours. The ⊿AUC-apoB did not differ among the four trials (**Table 5**).

The ⊿apoB48 in the F55G45 and G100 trials were significantly increased at 1 hour compared with each fasting value (p<0.05 each), but at 2 hours in the F100 and F90G10 trials (p<0.05 each). The ⊿apoB48 peaked at 2 hours in the G100 and at 4 hours in the other trials. At 4 hours, the ⊿apoB48 in the F100 trial tended to be higher than that observed in the G100 trial (p=0.09). In all but the G100 trial, the ⊿apoB48 did not return to the fasting values at 6 hours. However, there was no significant difference in the ⊿AUC-apoB48 among the four trials (**Table 5**).

Discussion

We previously demonstrated that the ingestion of fructose combined with fat led to a significantly higher rise (with a delayed peak) in serum concentrations of TG, RLP-TG, RemL-C and apoB48 compared to the ingestion of glucose with fat, suggesting a delay in the removal of intestinal TRL, namely CM and CM-R [8]. In that study, we examined the effect of pure fructose with fat on postprandial lipoprotein metabolism. However, in daily dietary habits, fructose is usually consumed with glucose as HFCS or sucrose, like that contained in colas and other

soft drinks. Therefore, in the present study, we examined the effect of mixed ingestion of fructose and glucose (as HFCS) combined with fat on postprandial lipoprotein and carbohydrate metabolism.

The major finding of the present study is that the ingestion of high-fructose- (but not glucose-) containing beverages combined with fat cream (F100 or F90G10 trial) exacerbated both exogenous (intestinal) and endogenous (hepatic) lipidemia postprandially, and that the ratio of fructose to glucose was a key factor of the metabolic disturbance.

Following the ingestion of a high-fructose-containing beverage with fat, the concentration of apoB48 was significantly increased after 2 hours, and the peak was delayed, compared with that observed following the ingestion of glucose with fat. This suggests delayed intestinal absorption of dietary fat and/or delayed secretion of CM from the intestine. It is possible that this finding depends on the difference in the absorption mechanisms of fructose and glucose because the transfer of fructose into the enterocytes by facilitated diffusion may be slower than that of glucose by active transport[16]. Moreover, in all but the G100 trial, the concentration of apoB48 did not return to baseline at 6 hours. These results suggest delayed intestinal absorption of dietary fat or secretion of CM from the intestine in addition to the delayed removal of CM and CM-R, consistent with our previous study[8].

The serum concentration of apoB(100) was decreased at 1-2 hours and returned to baseline at 4 hours in the all trials, suggesting transient suppression of secretion or stimulation of the removal of VLDL. However, in the absence of the increase of apoB(100) concentration, the concentration of hTRL-TG was significantly increased at 4 hours except in the G100 trials. Because one apoB100 molecule is present in each VLDL particle, the relationship between the hepatic VLDL-TG and VLDL-apoB100 secretion allows estimation of the TG content in the VLDL particles secreted by the liver[17]. The results indicate an increase in hepatic TRL particle size without a concurrent increase in their number following the ingestion of a fructose- (but not glucose-) containing beverage combined with fat. Although postprandial dyslipidemia was previously regarded as the consequence of delayed TRL removal, emerging evidence implicates intestinal[18] and hepatic[19] overproduction of TRL as major contributors to postprandial dyslipidemia. The increase of hepatic VLDL secretion may also inhibit the metabolism of CM and CM-R by the liver, by competing with the exogenous lipoprotein metabolism, because the endogenous and exogenous pathways share the same lipolytic enzyme, lipoprotein lipase (LPL), on the capillary endothelium of extrahepatic tissues, particularly adipose tissue and

skeletal muscle[20].

It has been reported that a single bolus of fructose rapidly increases hepatic de novo lipogenesis[21] and enhances VLDL-TG production[22]. Additionally, serum insulin concentration was lower following the ingestion of fructose with fat as compared to glucose, and serum FFA concentration tended to be less suppressed following the ingestion of fructose rather than glucose. Insulin acutely suppresses FFA release into the circulation from adipose tissue and reduces the flow of substrates for VLDL assembly in the liver[23, 24]. Because insulin and glucose also increase LPL activity in adipose tissue[25], the lower insulin and glucose excursion observed following the ingestion of fructose compared with glucose ingestion may have resulted in reduced activation of LPL, thus leading to delayed lipolysis of TG in CM and VLDL[26], and eventually, to postprandial prolonged increase in TRL concentration.

In the present study, serum lactate concentration increased at 1 and 2 hours in the F100 and F90G10 trials, but not in the F55G45 and G100 trials, suggesting that when the ratio of fructose to glucose is much higher, serum lactate concentration may be increased. This increased lactate production may occur because fructokinase activity was increased, the rate-limiting step for glycolysis (phosphofructokinase) was bypassed, and the pyruvate kinase activity was stimulated by the accumulation of fructose-1-phosphate[27]. The increase in serum concentrations of β-HB and FFA at 4 and 6 hours following the ingestion of fructose-containing beverages tended to be smaller than that observed in a glucose-containing beverage, consistent with Chong et al[26]. β-HB is oxidized following the oxidation of FFA in the liver. Thus, the lower serum FFA concentration is consistent with the smaller increase in serum β-HB concentration.

The present results suggest that the ingestion of a high amount of fructose stimulated the synthesis of fatty acids, and then TG-richer, large VLDL in the liver. They also suggest that when the same total amount of sugar (fructose and/or glucose) ingested, the higher the ratio of fructose to glucose, the greater the metabolic disturbance. The lower insulin excursion observed following fructose compared with glucose may also have resulted in reduced activation of LPL and led to delayed TG removal from CM and VLDL. In addition, the lower insulin excursion may have increased the leakage of FFA from the adipose tissue and its supply to the liver. Altogether, we conclude that high amounts of fructose ingestion exacerbate the postprandial rise not only of exogenous lipoproteins (CM and CM-R) but also of endogenous lipoproteins (VLDL and VLDL-R).

In summary, we demonstrated that simultaneous ingestion of a high-fructose-containing beverage and fat cream caused both exogenous and endogenous lipidemia postprandially, even in young healthy women. It may thus have even more unfavorable effects on the health of subjects with metabolic syndrome or obesity. These results suggest that even a single ingestion of a high-fructose-containing beverage combined with fat induced a rapid lipogenesis in the liver, enhanced the secretion of TG-richer VLDL, and delayed removal of CM and CM-R from the circulation, However, because of the relatively small number of subjects studied, the present results should be interpreted with caution. At present, there are no reliable data on the upper limit of the intake of fructose and HFCS for maintaining health in Japanese individuals[28] and the influence of fructose and HFCS ingestion on health remains to be clarified. However, it may be advisable to avoid fructose- or HFCS-containing beverages as much as possible in daily life, even for young healthy women.

Conclusion

A single ingestion of a high-fructose-containing beverage combined with fat cream caused postprandial exacerbation of both exogenous and endogenous lipidemia, even in young healthy women. The ratio of fructose to glucose was a determinant of metabolic disturbance when sugar load was equicaloric.

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Table 1. Anthropometric and clinical characteristics.

Trial	F100	F90G45	F55G45	G100
Age (years)	20.7 ± 0.2			
Height (cm)	158.7 ± 1.3			
Weight (kg)	52.0 ± 1.7	51.9 ± 1.8	51.5 ± 1.7	51.9 ± 1.7
BMI (kg/m^2)	20.7 ± 0.8	20.6 ± 0.8	20.5 ± 0.8	20.7 ± 0.8
Waist (cm)	70.9 ± 1.8	70.9 ± 1.6	70.7 ± 1.6	70.8 ± 1.7
W/H	0.80 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.80 ± 0.01
VFA (cm^2)	26.5 ± 4.5	28.2 ± 4.6	26.9 ± 4.2	25.3 ± 3.7
HbA1c (NGSP) (%)	5.3 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
HOMA-IR	1.3 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.2
TC (mg/dL)	162.7 ± 6.9	158.6 ± 6.4	166.3 ± 5.9	163.0 ± 6.4
LDL-C (mg/dL)	88.4 ± 4.4	85.7 ± 3.7	92.3 ± 3.9	88.0 ± 3.4
HDL-C (mg/dL)	63.8 ± 3.4	61.1 ± 3.5	63.8 ± 2.8	62.6 ± 4.0
Lp(a) (mg/dL)	18.5 ± 8.8			
apoAI (mg/dL)	149.2 ± 5.5	145.9 ± 6.0	148.4 ± 4.8	149.8 ± 7.0
apoAII (mg/dL)	25.4 ± 1.0	25.3 ± 0.9	25.3 ± 0.8	25.8 ± 1.0
apoCII (mg/dL)	2.4 ± 0.2	2.6 ± 0.3	2.5 ± 0.2	2.6 ± 0.3
apoCIII (mg/dL)	7.0 ± 0.5	6.9 ± 0.5	7.1 ± 0.5	7.3 ± 0.6
apoE (mg/dL)	3.9 ± 0.2	4.1 ± 0.3	4.0 ± 0.2	4.0 ± 0.2
SBP (mmHg)	98.2 ± 1.9	99.9 ± 1.6	98.7 ± 1.9	96.9 ± 1.2
DBP (mmHg)	61.8 ± 1.5	62.9 ± 1.1	60.6 ± 1.5	61.9 ± 1.5
PR (beats/min)	66.8 ± 2.0	68.6 ± 2.0	67.3 ± 1.6	67.4 ± 1.4

All values are presented as mean ± SEM.

SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: pulse rate

Table 2. The values of fasting and postprandial fructose, glucose, insulin, FFA, β-HB and lactate.

	Time (h)	0	0.5	1	2	4	6
Fructose (mg/dL)	F100	0.72 ± 0.06	4.25 ± 0.74*	5.48 ± 0.54*	3.99 ± 0.50*	0.79 ± 0.06	0.83 ± 0.08
	F90G10	0.63 ± 0.05 #	4.64 ± 0.64*	4.74 ± 0.54*	2.75 ± 0.25* #	0.98 ± 0.12* #	0.85 ± 0.06
	F55G45	0.92 ± 0.07	3.02 ± 0.52* # #	3.02 ± 0.29* # #	2.08 ± 0.23* # #	0.85 ± 0.09	0.69 ± 0.07
	G100	0.62 ± 0.08	0.78 ± 0.09	0.54 ± 0.08	0.86 ± 0.06	0.65 ± 0.07	0.74 ± 0.07
Glucose (mg/dL)	F100	84.0 ± 1.5	88.9 ± 1.9	86.4 ± 1.7	87.8 ± 1.7	84.1 ± 1.4	80.8 ± 1.8
	F90G10	85.0 ± 1.1	91.5 ± 1.8*	87.3 ± 1.6	87.3 ± 1.2	83.0 ± 1.1	80.1 ± 1.1*
	F55G45	86.3 ± 1.2	101.7 ± 4.3	92.6 ± 4.0	89.6 ± 2.0	82.9 ± 1.7	80.4 ± 1.3*
	G100	85.6 ± 1.5	106.7 ± 4.5*	99.1 ± 4.9	93.0 ± 3.9	82.9 ± 1.7	80.1 ± 1.4*
Insulin (μU/mL)	F100	6.23 ± 0.75	13.93 ± 1.44* #	12.57 ± 1.98* #	9.69 ± 0.96*	4.12 ± 0.50*	3.29 ± 0.54*
	F90G10	5.71 ± 0.54	19.31 ± 2.42*	12.55 ± 1.52* #	10.39 ± 1.16*	3.54 ± 0.34*	3.33 ± 0.56*
	F55G45	6.48 ± 0.70	30.84 ± 6.53*	18.80 ± 3.19* #	9.82 ± 1.04*	5.03 ± 1.06	3.89 ± 0.73*
	G100	5.79 ± 0.89	36.98 ± 6.32*	31.31 ± 6.10*	11.87 ± 2.00*	3.64 ± 0.41*	3.28 ± 0.42*
FFA (μmol/L)	F100	426.3 ± 38.4		266.6 ± 44.3	256.2 ± 34.9*	608.8 ± 46.1*	776.3 ± 38.7*
	F90G10	495.7 ± 49.4		255.4 ± 32.2*	256.6 ± 35.4*	605.3 ± 34.8	730.3 ± 32.0*
	F55G45	511.8 ± 50.9		255.6 ± 36.2*	280.2 ± 29.5*	700.2 ± 39.8*	852.3 ± 48.1*
	G100	460.3 ± 52.4		173.8 ± 19.5*	178.7 ± 22.5*	673.3 ± 45.3*	825.4 ± 47.2*
β-HB (μmol/L)	F100	63.4 ± 9.6		49.9 ± 9.5	33.0 ± 3.7	175.8 ± 35.7*	294.5 ± 41.1*
	F90G10	73.4 ± 15.2		47.4 ± 9.3	31.7 ± 3.3*	156.0 ± 22.4*	288.4 ± 34.8*
	F55G45	87.3 ± 26.0		54.7 ± 10.9	46.3 ± 8.0	232.3 ± 31.9*	355.8 ± 42.2*
	G100	69.2 ± 17.8		54.7 ± 11.2	35.4 ± 4.4	219.4 ± 36.4*	377.6 ± 80.0*
Lactate (mg/dL)	F100	9.3 ± 0.6		15.9 ± 1.8*	13.8 ± 1.0* #	8.4 ± 0.5	8.3 ± 0.6
	F90G10	9.9 ± 0.6		15.9 ± 1.3*	12.2 ± 0.6	8.7 ± 0.6	8.4 ± 0.5
	F55G45	10.2 ± 0.6		13.9 ± 1.0	11.9 ± 0.8	8.9 ± 0.5	8.8 ± 0.4
	G100	10.5 ± 1.0		11.3 ± 0.7	9.9 ± 0.5	9.0 ± 0.5	8.8 ± 0.5

All values are presented as mean ± SEM. *p<0.05 compared to the fasting value in each trial. #p<0.05 compared between the trials.

Table 3. The values of fasting and postprandial TG, hTRL-TG, RLP-TG, RemL-C, apoB and apoB48.

	Time (h)	0	1	2	4	6
TG (mg/dL)	F100	52.3 ± 3.8	55.4 ± 3.8	64.3 ± 4.6*	94.8 ± 10.3*	66.3 ± 4.8*
	F90G10	59.3 ± 8.6	60.6 ± 7.9	70.8 ± 9.5*	101.2 ± 17.5*	68.7 ± 8.7
	F55G45	51.6 ± 5.6	54.6 ± 4.5	63.8 ± 4.4*	76.2 ± 10.2*	54.9 ± 5.7
	G100	62.3 ± 5.0	67.7 ± 4.5	78.2 ± 6.7	79.3 ± 8.6	60.4 ± 5.2
hTRL-TG (mg/dL)	F100	39.6 ± 4.5	43.8 ± 4.2	48.9 ± 4.2	68.1 ± 7.5*	50.2 ± 4.8*
	F90G10	46.8 ± 7.7	48.3 ± 7.5	53.1 ± 8.2*	69.9 ± 9.7*	52.0 ± 7.4
	F55G45	40.7 ± 6.0	43.0 ± 4.9	46.4 ± 4.6	55.2 ± 7.8*	41.5 ± 5.4
	G100	52.1 ± 5.8	56.2 ± 5.4	55.6 ± 5.1	58.2 ± 6.8	44.8 ± 4.6
RLP-TG (mg/dL)	F100	8.6 ± 0.9	11.2 ± 1.1	17.5 ± 2.2*	30.7 ± 4.7*	15.5 ± 1.7*
	F90G10	9.4 ± 1.3	11.3 ± 1.4	18.2 ± 2.6*	32.3 ± 9.0*	14.7 ± 2.3*
	F55G45	8.5 ± 0.7	12.1 ± 0.8*	18.5 ± 1.7*	22.5 ± 4.0*	12.0 ± 0.9*
	G100	9.6 ± 0.9	13.9 ± 1.0*	23.5 ± 3.4*	21.1 ± 3.3*	13.7 ± 1.6
RemL-C (mg/dL)	F100	3.7 ± 0.3	3.9 ± 0.4	4.2 ± 0.4	6.1 ± 0.7*	5.2 ± 0.5*
	F90G10	4.5 ± 1.0	4.4 ± 0.9	4.7 ± 0.9	6.4 ± 1.2*	5.7 ± 1.0*
	F55G45	4.0 ± 0.6	4.0 ± 0.5	4.3 ± 0.4	5.4 ± 0.7*	4.8 ± 0.7*
	G100	4.5 ± 0.4	4.7 ± 0.3	4.7 ± 0.4	5.3 ± 0.5	4.7 ± 0.5
apoB (mg/dL)	F100	63.2 ± 2.8	63.7 ± 1.7	61.3 ± 2.6*	63.1 ± 2.7	63.5 ± 2.5
	F90G10	62.6 ± 2.5	60.6 ± 2.5*	60.2 ± 2.4*	62.0 ± 2.6	62.5 ± 2.6
	F55G45	65.0 ± 2.8	62.5 ± 2.4*	62.3 ± 2.6*	64.1 ± 2.6	64.4 ± 2.8
	G100	64.5 ± 2.2	62.3 ± 1.9*	61.0 ± 1.8*	63.4 ± 1.8	63.9 ± 2.0
apoB48 (μg/mL)	F100	2.3 ± 0.3	2.9 ± 0.3	4.0 ± 0.5*	5.2 ± 0.6*	3.4 ± 0.2*
	F90G10	2.4 ± 0.6	3.0 ± 0.5	4.0 ± 0.6*	5.1 ± 1.1*	3.6 ± 0.6*
	F55G45	2.1 ± 0.1	3.2 ± 0.4*	3.8 ± 0.3*	4.1 ± 0.5*	3.3 ± 0.4*
	G100	2.7 ± 0.4	4.0 ± 0.6*	4.4 ± 0.5*	3.9 ± 0.6*	3.2 ± 0.4

All values are presented as mean ± SEM. *$p<0.05$ compared to the fasting value in each trial.

Table 4. The parameters before and after beverage ingestion.

	Time (h)	0	1	2	4	6
⊿TG (mg/dL)	F100	0.0 ± 0.0	3.1 ± 1.7	11.9 ± 3.4*	42.5 ± 7.0*	14.0 ± 2.4*
	F90G10	0.0 ± 0.0	1.3 ± 1.7	11.6 ± 3.0*	41.9 ± 9.9*	9.4 ± 4.5
	F55G45	0.0 ± 0.0	3.0 ± 2.0	12.3 ± 3.1*	24.6 ± 5.9*	3.3 ± 2.2
	G100	0.0 ± 0.0	5.3 ± 2.5	15.8 ± 5.9	16.9 ± 5.8	-1.9 ± 4.7
⊿hTRL-TG (mg/dL)	F100	0.0 ± 0.0	4.3 ± 2.0	9.3 ± 2.9	28.5 ± 4.5*	10.6 ± 1.5*
	F90G10	0.0 ± 0.0	1.5 ± 1.0	6.3 ± 1.8*	23.1 ± 3.3*	5.2 ± 3.9
	F55G45	0.0 ± 0.0	2.3 ± 1.5	5.8 ± 2.0	14.5 ± 2.8*	0.8 ± 2.4
	G100	0.0 ± 0.0	4.1 ± 1.8	3.5 ± 3.5	6.1 ± 4.1	-7.3 ± 4.8
⊿RLP-TG (mg/dL)	F100	0.0 ± 0.0	2.6 ± 1.2	8.9 ± 2.3*	22.1 ± 4.2*	6.9 ± 1.3*
	F90G10	0.0 ± 0.0	1.9 ± 1.2	8.8 ± 1.7*	23.0 ± 7.8*	5.3 ± 1.9*
	F55G45	0.0 ± 0.0	3.5 ± 0.8*	9.9 ± 2.0*	14.1 ± 3.5*	3.6 ± 0.7*
	G100	0.0 ± 0.0	4.4 ± 1.5*	14.0 ± 3.5*	11.6 ± 2.9*	4.1 ± 1.6
⊿RemL-C (mg/dL)	F100	0.00 ± 0.00	0.14 ± 0.16	0.49 ± 0.24	2.32 ± 0.40*	1.46 ± 0.17*
	F90G10	0.00 ± 0.00	-0.04 ± 0.12	0.28 ± 0.16	1.98 ± 0.33*	1.25 ± 0.30*
	F55G45	0.00 ± 0.00	0.02 ± 0.15	0.29 ± 0.18	1.44 ± 0.23*	0.82 ± 0.13*
	G100	0.00 ± 0.00	0.20 ± 0.15	0.28 ± 0.22	0.80 ± 0.31	0.24 ± 0.30
⊿apoB (mg/dL)	F100	0.0 ± 0.0	0.50 ± 1.79	-1.92 ± 0.58*	-0.08 ± 0.53	0.33 ± 0.64
	F90G10	0.0 ± 0.0	-2.00 ± 0.44*	-2.42 ± 0.54*	-0.58 ± 0.56	-0.08 ± 0.45
	F55G45	0.0 ± 0.0	-2.50 ± 0.60*	-2.67 ± 0.69*	-0.92 ± 0.42	-0.58 ± 0.47
	G100	0.0 ± 0.0	-2.17 ± 0.46*	-3.50 ± 0.51*	-1.08 ± 0.57	-0.58 ± 0.69
⊿apoB48 (μg/mL)	F100	0.0 ± 0.0	0.58 ± 0.24	1.63 ± 0.35*	2.88 ± 0.45*	1.11 ± 0.11*
	F90G10	0.0 ± 0.0	0.54 ± 0.23	1.58 ± 0.22*	2.68 ± 0.57*	1.19 ± 0.31*
	F55G45	0.0 ± 0.0	1.02 ± 0.38*	1.70 ± 0.31*	1.97 ± 0.42*	1.18 ± 0.28*
	G100	0.0 ± 0.0	1.34 ± 0.30*	1.73 ± 0.28*	1.29 ± 0.36*	0.53 ± 0.29

All values are presented as mean ± SEM. *p<0.05 compared to the fasting values. #p<0.05 compared between the trials.

Table 5. The incremental area under the curve (⊿AUC) for TG, hTRL-TG, RLP-TG, RemL-C, apoB and apoB48.

Trial	⊿AUC-TG (mg・h/dL)	⊿AUC-hTRL-TG (mg・h/dL)	⊿AUC-RLP-TG (mg・h/dL)	⊿AUC-RemL-C (mg・h/dL)	⊿AUC-apoB (mg・h/dL)	⊿AUC-apoB48 (μg・h/mL)
F100	120.0 ± 15.1	85.8 ± 13.1	67.1 ± 9.9	7.0 ± 1.3	-2.2 ± 3.6	9.9 ± 1.0
F90G10	112.0 ± 19.7	62.2 ± 7.9	66.3 ± 16.9	5.6 ± 0.7	-6.9 ± 2.6	9.5 ± 1.2
F55G45	73.9 ± 8.9	40.8 ± 5.2	49.9 ± 6.4	4.2 ± 0.3	-8.9 ± 2.5	8.7 ± 1.2
G100	61.0 ± 15.8	14.2 ± 13.9	52.4 ± 8.2	2.5 ± 1.0	-10.2 ± 2.7	7.1 ± 1.0

All values are presented as mean ± SEM. *p<0.05

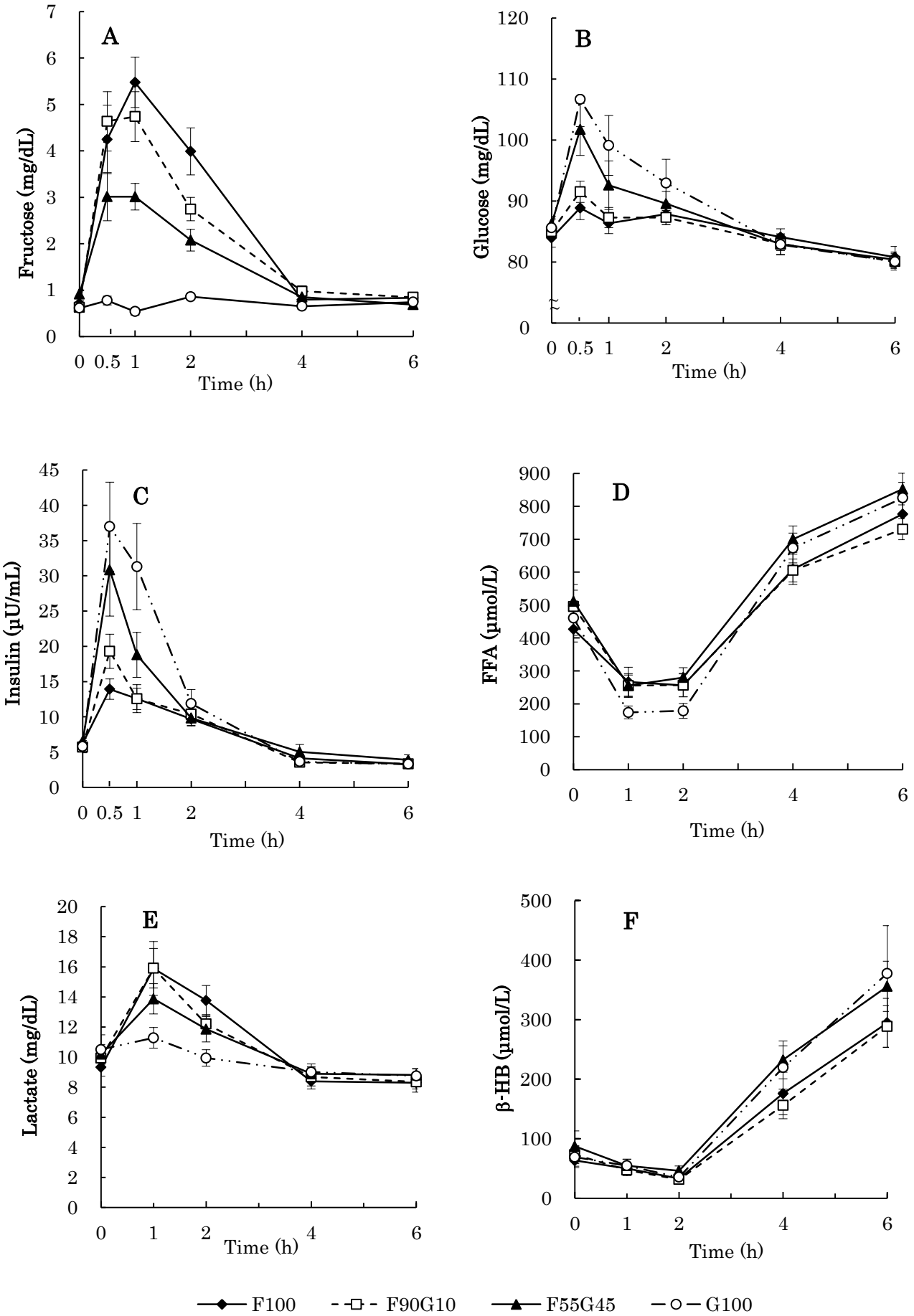

Fig. 1. Postprandial Fructose (A), Glucose (B), Insulin (C), FFA (D), Lactate (E) and β-HB (F). The values are presented as mean ± SEM.

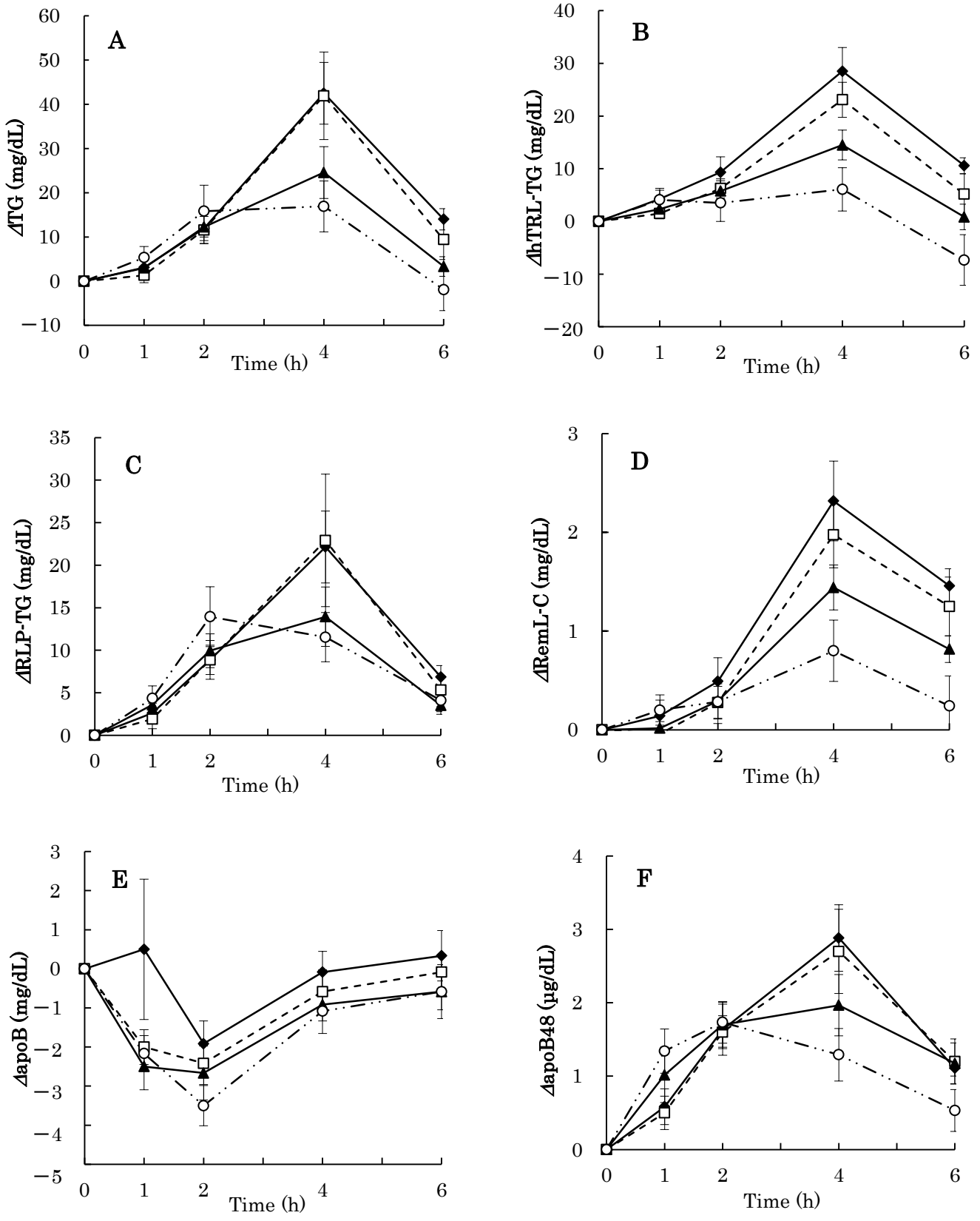

Fig. 2. Postprandial *Δ*TG (A), *Δ*hTRL-TG (B), *Δ*RLP-TG (C), *Δ*RemL-C (D), *Δ*apoB (E) and *Δ*apoB48 (F). The values are presented as mean ± SEM.

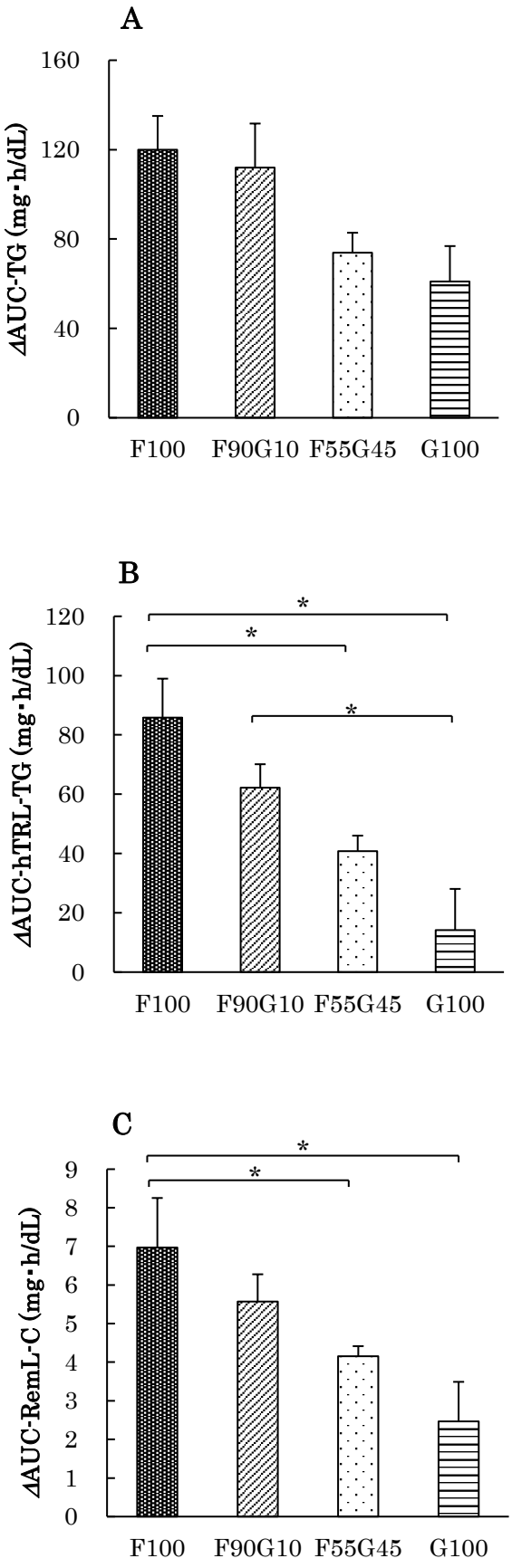

Fig. 3. The incremental area under the curve (*Δ*AUC) for TG (A), hTRL-TG (B) and RemL-C (C).
The values are presented as mean ± SEM. **p*<0.05

Chapter 3

Ingestion of a large volume of water disturbs fructose absorption
in young healthy women
(Published in Journal for the Integrated Study of Dietary habits 2013; 24: 92-97)

Abstract

Aim: To examine the absorption of fructose by measuring the concentration of breath hydrogen (BH) after the intake of fructose-containing beverages.

Methods: Seventeen young healthy Japanese women were enrolled as participants and were studied on four occasions. At each session, subjects ingested one of four beverages after a 12-hour overnight fast. The beverages were prepared as follows; 10%F (Control): 25 g of fructose with 250 mL of water, 5%F: control beverage and 250 mL of water at 0 hours, totally 25 g of fructose in 500 mL of water, 10%F+0.5hW: control beverage and 250 mL of water at 0.5 h, 10%F+1hW: control beverage and 250 mL of water at 1 hour. Abdominal symptoms were recorded by the visual analog scales (VAS).

Results: BH in the 10%F, 10%F+0.5hW and 10%F+1hW trials was significantly increased at 45 or 60 minutes, and peaked at 75 or 90 minutes, and returned to the baseline level at 120 or 135 minutes. BH in the 5%F trial was significantly increased at 45 minutes and peaked at 75 minutes, but did not return to the baseline level at 180 minutes. The ΔAUC-BH in the 5%F trial was significantly larger than that in the 10%F trial. Abdominal symptoms occurred in all trials, however, there were no significant differences in the strength of each symptom and the number.

Conclusions: Fructose malabsorption and gastrointestinal symptoms were caused in all trials. Of these, BH was significantly increased in the 5% trial, suggesting that, even at a low concentration, fructose absorption may be disturbed by the intake of a large volume of water.

Introduction

High fructose corn syrup (HFCS) was developed industrially as an alternative to sucrose in the USA and came into wide usage during the period between 1970 and 1985[1]. In Japan, the consumption of HFCS has been increasing since 1980, while that of sucrose has decreased. The most common use of HFCS is in soft drinks[2].

Many studies have shown the relation between the intake of high-fructose-containing beverages and fructose malabsorption[3, 4]. High amounts of fructose often induce gastrointestinal symptoms, such as bloating, abdominal pain and diarrhea[5, 6]. Unabsorbed fructose is transferred from the small intestine to the colon, and hydrogen is produced by colonic bacteria[5]. Part of the hydrogen produced in the colon diffuses into the bloodstream and is excreted from the breath via the lung. Therefore, the quantification of hydrogen in the breath can be used to estimate incomplete fructose absorption[7]. In a previous study, we showed that the intestinal transit time of fructose was approximately 60 minutes[8]. Accordingly, in the present study, we examined the effect of water intake at two different times (at 0.5 hours or 1 hour) after the ingestion of a fructose-containing beverage.

The highest consumers of fructose are adolescents and young adults in the USA[9], and this is probably also the case in Japan. Therefore, we enrolled young healthy Japanese women as subjects and examined the absorption of fructose-containing beverages by measuring the concentration of breath hydrogen (BH) after the intake of fructose-containing beverages.

Aim

To elucidate the factors related to the absorption of fructose by measuring the concentration of BH in young healthy women.

Methods

Subjects

Seventeen young healthy Japanese women with normal weight (BMI 18.5≤–<25) who did not release methane from the breath were enrolled as participants. Subjects were non-smokers, were not suffering from any apparent acute or chronic illness, and were not taking antibiotics during one month before the experiment. This study was approved by the Institutional Review Board of Sugiyama Jogakuen

University School of Life Studies, and each subject gave written informed consent for study participation.

Anthropometric and body composition measurement

Body weight and height were measured by standard methods. The waist circumference was assessed as the abdominal girth at the level of the umbilicus, and the hip was measured at the level of the greater trochanters. The waist-to-hip ratio (W/H) was calculated. Body composition including visceral fat area (VFA) was analyzed by an eight-polar bioelectrical impedance method, using InBody720 (Biospace, Tokyo, Japan).

Fructose load test & experimental design

Each subject was studied on four occasions in a randomized crossover design. At each session, subjects ingested one of four beverages after a 12-hours overnight fast. The beverages were prepared as follows: 10%F (Control): 25 g of fructose (Nisshin Seito, Tokyo, Japan) with 250 mL of water; 5%F: control beverage and 250 mL of water at 0 hours, totally 25 g of fructose in 500 mL of water; 10%F+0.5hW: control beverage and 250 mL of water at 0.5 hours; 10%F+1hW: control beverage and 250 mL of water at 1 hours.

During the test, subjects avoided exercise, eating, and access to water. The experiments were performed at an interval of one week. Subjects avoided intake of alcohol, dairy products, and high-dietary fiber foods such as legumes, mushrooms, root vegetables, and seaweed for dinner on the day before the experiment, because these foods and liquids may affect BH excretion[8].

Measurement of breath hydrogen concentration

Fructose malabsorption was measured by a breath hydrogen/methane analyzer (BGA-1000D, Laboratory for Breath Biochemistry Nourishment Metabolism, Nara, Japan). The terminal-expiratory gas was collected at the baseline (0 minutes) and every 15 minutes during a 180-minutes period, using the gas collection bag. The gas samples were taken with subjects in the sitting position. Changes in BH were calculated as the difference from the baseline mean value (as 0 at 0 hours) and shown as ΔBH. Incomplete fructose absorption was defined as a rise of ΔBH $\geq$20 ppm, which is highly specific for identifying carbohydrate malabsorption[5, 10]. Changes in the BH were quantified by calculating the incremental area under the curve (ΔAUC), which was defined as the difference between the area under the curve from 0

minutes to 180 minutes and the area below the baseline (0 minutes).

Estimation of abdominal symptoms

Abdominal symptoms (abdominal pain, bloating, flatulence, and borborygmus) were recorded by the visual analog scales (VAS, 0 to 10 cm) at baseline (0 minutes) and every 15 minutes after the intake of test beverages during a 180-minutes test period[5, 11]. The results were expressed as the sum of the individual scores of abdominal symptoms.

Statistics

All data are expressed as means ± SEM. Statistical analyses were performed using StatView ver. 5.0 (SAS Institute, NC, USA). Differences in the time-course changes from the baseline values were analyzed using repeated measure one-way ANOVA, followed by the post-hoc test of Fisher's PLSD. The measured value differences at each time point in the 10%F vs. 5%F trials and the 10%F+0.5hW vs. 10%F+1hW trials were assessed by an unpaired t-test. $p<0.05$ was considered to be significant in all analyses.

Results

The physical characteristics of subjects are shown in **Table 1**. There were no significant differences in any of the physical characteristics in the four trials.

Changes in BH in the four trials are shown in **Table 2, Fig. 1**. BH in the 10%F, 10%F+0.5hW and 10%F+1hW trials were significantly increased at 45 or 60 minutes ($p<0.05$ each), and peaked at 75 or 90 minutes, and returned to the baseline level at 120 or 135 minutes. BH in the 5%F trial was significantly increased at 45 minutes ($p<0.05$) and peaked at 75 minutes, but did not return to the baseline level at 180 minutes.

ΔBH in the four trials are shown in **Fig. 2**. ΔBH in the 5%F trial was significantly higher than that in the 10%F trial at 150 and 165 minutes ($p<0.05$ each). ΔBH in the 10%F+0.5hW vs. 10%F+1hW trials was not significantly different at any time point. The numbers of subjects with ΔBH $\geq$20 ppm were as follows: 10 in the 5%F trial, 8 in the 10%F+1hW trial, 5 in the 10%F trial and 3 in the 10%F+0.5hW trial.

The ΔAUC-BH is presented in **Fig. 3**. The ΔAUC-BH in the 5%F trial was significantly larger than that in the 10%F trial ($p<0.05$). The ΔAUC-BH in the 10%F+0.5hW vs. 10%F+1hW trials were not significantly different.

Changes of abdominal symptoms in the 4 trials are shown in **Fig. 4A-D**. The sum

of the individual abdominal symptoms score is presented in **Fig. 5**. Asymptomatic subjects were as follows: 5 in the 10%F and 10%F+0.5hW trials, and 4 in the 5%F and 10%1hW trials. However, there were no significant differences in the strength of each symptom and the number in the four trials. The number of cases of acute diarrhea was as follows: 2 in the 10%F+0.5hW trial and 1 in each of the other trials, and occurred at 45–105 minutes after fructose ingestion (**Table 3**).

Discussion

The frequency of fructose malabsorption increases with higher amounts and concentrations of fructose intake[3, 4]. Changes in BH after the ingestion of 10% fructose beverage containing 25 g of fructose in 10 healthy adult subjects showed 5 subjects with fructose malabsorption. i.e., 50%[3]. In the present study, the ΔAUC-BH in the 5%F trial was significantly larger than that in the 10%F trial, suggesting that a large volume of water suppressed fructose absorption even at the lower concentration. Our current hypothesis on fructose malabsorption in the 5%F trial is presented in **Fig. 6**. The ΔAUC-BH in the 5%F trial is suggested to have been increased by the effect of the increased generation of gas in the colon. There was no significant difference in the 10%F+0.5hW vs. the 10%F+1hW trials, suggesting that fructose absorption was not affected by the water taken after the ingestion of fructose-containing beverage, and ingested fructose rapidly moved into the colon within 0.5 hours.

Fructose is transported into the enterocytes through a specific fructose transporter, GLUT5, located at the intestinal membrane. Absorption of fructose by facilitated diffusion, which does not need adenosine 5'-triphosphate, differs from that of glucose[12, 13]. The contact of fructose with the intestinal brush-border membrane may also have been decreased by a large amount of water, and the transfer of fructose into the enterocytes by facilitated diffusion may have become slower. It is likely that unabsorbed fructose, together with a large amount of water, rapidly drifted into the colon from the small intestine and increased the production of hydrogen, methane, and fatty acids by bacterial anaerobic fermentation[5].

The greatest number of subjects with ΔBH $\geq$20 ppm, defined as incomplete fructose absorption[10], was found in the 5%F trial. Nevertheless, there was no significant difference in the VAS of abdominal symptoms among the four trials, and there was no correlation between the ΔAUC-BH and each gastrointestinal symptom. These symptoms are attributed to intestinal and/or colonic distention due to the

osmotic effect of incompletely absorbed fructose, rather than the effect of gases produced by colonic bacterial fermentation[5]. Quantification of the fructose malabsorption is difficult to estimate by the breath test only. In this study, although BH and abdominal symptoms had great differences among individuals, the breath test provided a valuable non-invasive diagnostic strategy.

In the present study, only fructose was ingested, but fructose is usually contained with glucose in soft drinks or foods, and fructose absorption is promoted by glucose[3, 8]. Sucrose is composed of fructose and glucose, and it is normally completely absorbed[14]. When the amount of fructose is greater than that of glucose, fructose malabsorption could be induced[3, 8]. The abnormality of ΔBH was less in the HFCS-55 (55% fructose and 45% glucose) ingestion compared with the ingestion of fructose only, and symptoms such as abdominal pain, bloating, and flatulence were not observed after the HFCS-55 ingestion[5].

HFCS is classified into three different types by the Japan Agricultural Standard[15], as follows: high-fructose liquid sugar (fructose ≥90%); fructose-glucose liquid sugar (fructose 50≤–<90%), and glucose-fructose liquid sugar (fructose <50%). The products in which HFCS are contained include soft drinks, which account for ca. 50% of the total, followed by milk beverages, seasoning, bread, and cold desserts[2]. Fructose malabsorption is likely to develop in infants, and the majority of infants experience fructose malabsorption after 0.5 g/kg of fructose ingestion[16]. HFCS is often added to fruit-flavored juice or sports drinks[17]. Even 100% apple or pear juice contains more fructose than glucose[18]. Thus, attention to the use of these beverages is necessary because of the low amounts of fructose that can be tolerated by babies and infants compared to adults. Also, patients with irritable bowel syndrome were reported to be sensitive to fructose and HFCS ingestion[5]. Ingestion of HFCS in healthy subjects did not cause the abdominal symptoms, but the patients with irritable bowel syndrome were more likely to develop abdominal symptoms[5].

There are many reports that fructose may have adverse effects on human health. Our previous study demonstrated that the ingestion of fructose with fat exacerbates postprandial lipidemia in young healthy women[19]. In the USA, many studies have shown the relation between the intake of sugar-sweetened beverages and weight gain[20]. Overconsumption of fructose- or HFCS-containing beverages may enhance the risk of metabolic syndrome, which is becoming a serious problem not only in the USA but also in Japan. The World Health Organization proposed that added sugars should provide no more than 10% of dietary energy[21]. However, the influence of intake of fructose and HFCS on health remains to be clarified, and very little data about the effects on the

Japanese people are available. For the present, there is no evidence-based data on the upper limit of the intake of fructose and HFCS for maintaining health in the dietary intake reference for Japanese[22]. Elucidation of the influence of fructose and HFCS ingestion is mandatory to protect the health of the Japanese population, and a strategy of dietary education for children and adolescents about this issue must be formulated as soon as possible in Japan.

Conclusion

The present study demonstrated that fructose-containing beverages are liable to cause gastrointestinal symptoms, and fructose absorption, even at a low concentration, may be disturbed by the intake of a large volume of water.

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Table 1. Anthropometric characteristics

	10%F	5%F	10%F+0.5hW	10%F+1hW
Age (years)	21.4 ± 0.1			
Height (cm)	158.7 ± 1.4			
Weight (kg)	51.2 ± 1.3	51.3 ± 1.3	51.3 ± 1.3	51.3 ± 1.3
BMI (kg/m^2)	20.3 ± 0.4	20.3 ± 0.4	20.3 ± 0.4	20.4 ± 0.4
Waist (cm)	69.9 ± 1.1	69.6 ± 1.0	70.0 ± 1.1	70.7 ± 1.2
Hip (cm)	89.6 ± 0.8	89.5 ± 0.8	89.6 ± 0.8	89.8 ± 0.9
W/H	0.80 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.80 ± 0.01
%Body fat (%)	28.4 ± 0.8	28.1 ± 0.9	28.3 ± 0.8	28.8 ± 0.9
VFA (cm^2)	39.7 ± 2.8	37.6 ± 2.3	39.2 ± 2.7	39.3 ± 2.6
SBP (mmHg)	103.3 ± 2.1	105.2 ± 1.3	102.9 ± 1.8	104.1 ± 1.6
DBP (mmHg)	68.1 ± 2.1	66.1 ± 1.7	66.0 ± 1.9	68.0 ± 1.6
PR (beats/min)	70.1 ± 2.8	72.0 ± 1.8	67.6 ± 2.2	69.6 ± 2.3

Values are presented as mean ± SEM.

SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: pulse rate

Table 2. Changes of BH concentration.

(ppm)

Time (min)	10%F	5%F	10%F+0.5hW	10%F+1hW
0	12.4 ± 3.8	12.5 ± 2.3	15.9 ± 3.2	9.8 ± 2.4
15	14.0 ± 3.8	14.9 ± 2.9	16.8 ± 3.2	12.4 ± 2.3
30	14.4 ± 3.3	19.7 ± 4.5	18.3 ± 4.2	13.5 ± 2.4
45	16.8 ± 3.5	31.4 ± 7.4*	20.7 ± 5.7	17.3 ± 3.6*
60	22.9 ± 5.2*	36.1 ± 7.8*	25.1 ± 7.9*	20.3 ± 3.9*
75	23.0 ± 6.0*	39.9 ± 8.8*	26.5 ± 8.3*	24.2 ± 4.4*
90	21.9 ± 5.4*	39.0 ± 7.7*	26.9 ± 8.2*	22.4 ± 4.0*
105	19.4 ± 5.9*	37.3 ± 8.8*	24.9 ± 7.3*	19.2 ± 3.4*
120	18.4 ± 5.3	35.1 ± 8.4*	25.6 ± 7.9*	17.6 ± 3.2*
135	17.4 ± 4.7	33.5 ± 8.1*	22.7 ± 5.3	15.5 ± 3.0
150	14.9 ± 3.8	33.1 ± 8.5*	21.9 ± 4.7	14.3 ± 2.7
165	13.5 ± 3.1	33.5 ± 9.2*	19.0 ± 3.3	14.8 ± 3.0
180	12.8 ± 3.1	28.6 ± 7.5*	17.1 ± 2.8	13.6 ± 2.9

Values are presented as the means ± SEM.

*p<0.05 compared with the baseline (0 min).

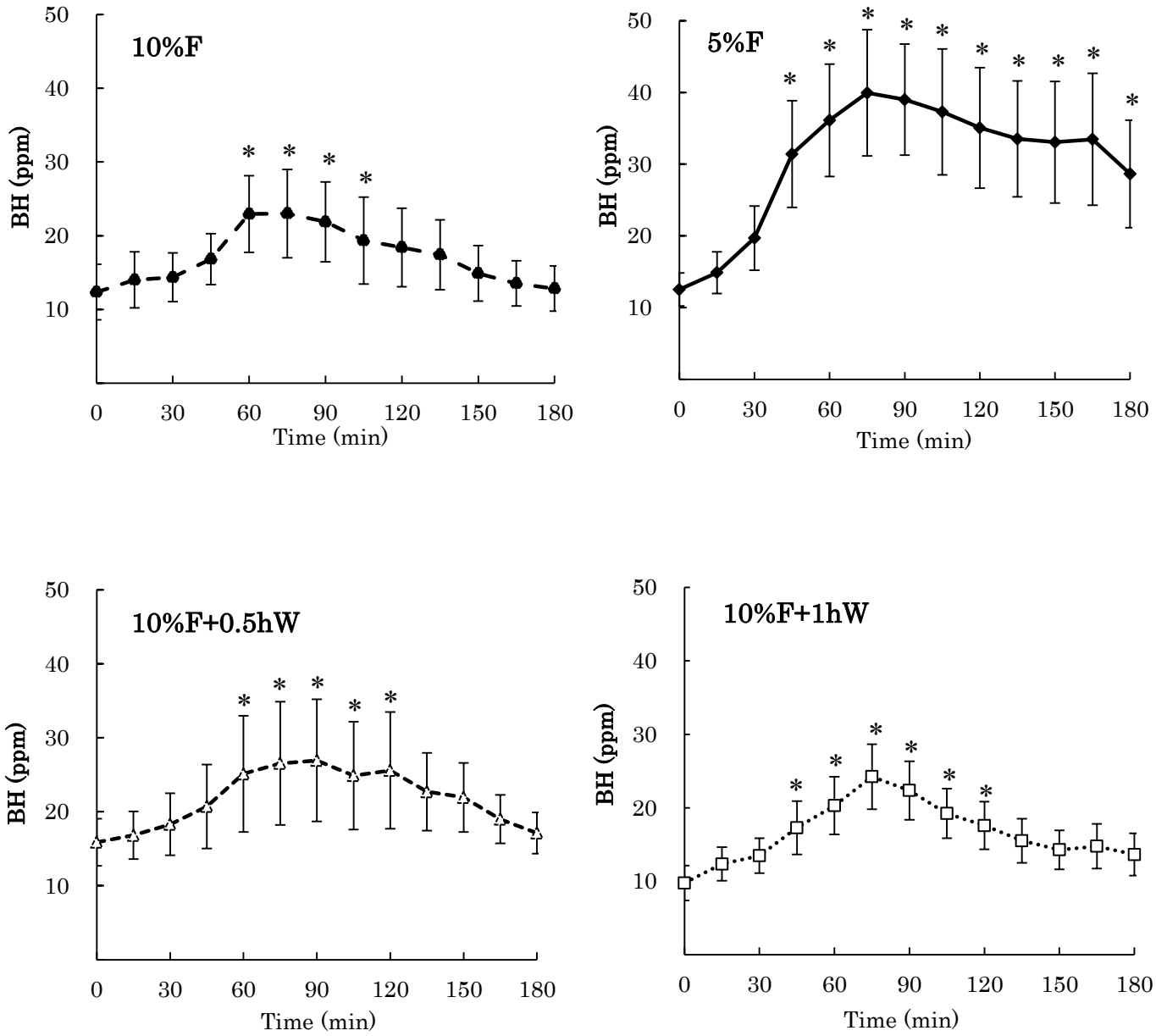


Fig. 1. Changes of BH concentration in the four trials.
Values are presented as mean ± SEM. $*p<0.05$ compared with the baseline value (0 minutes).

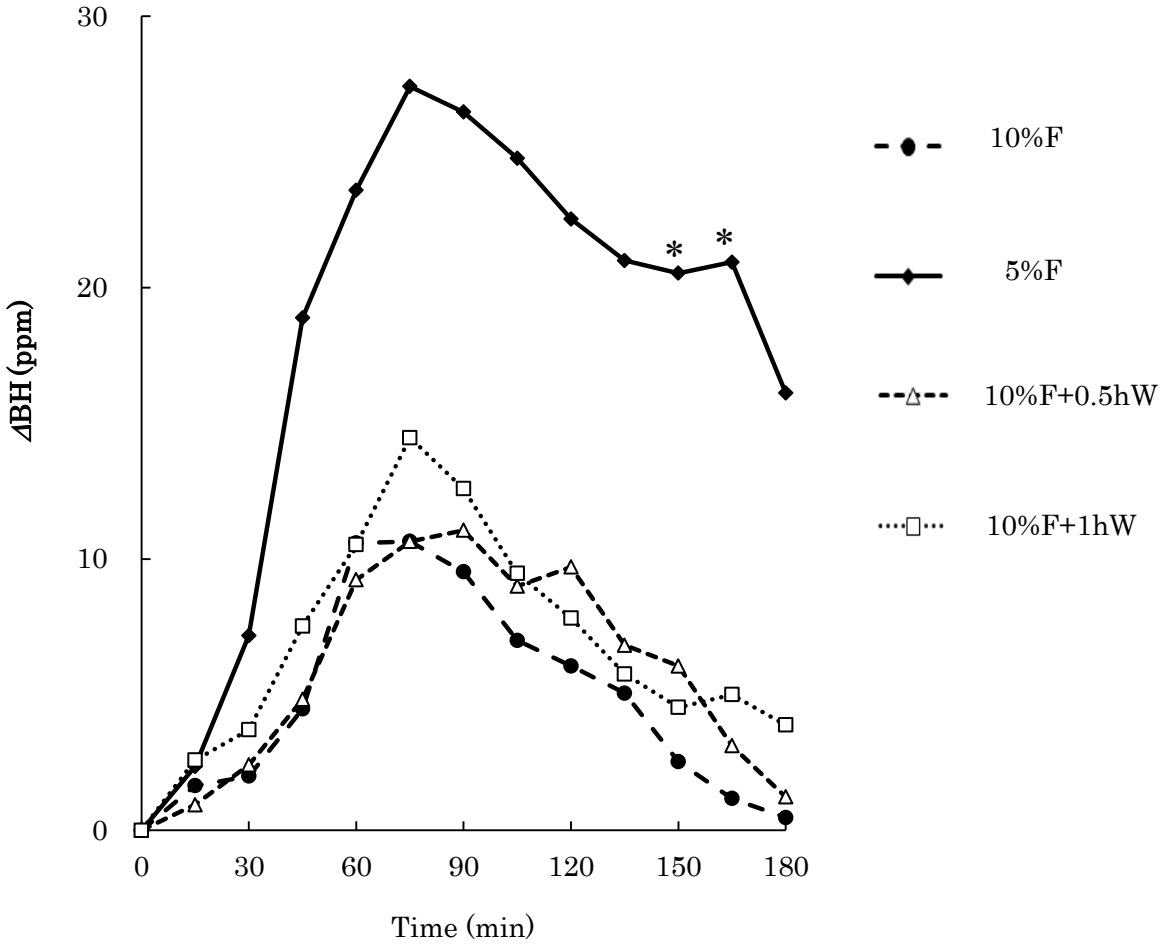
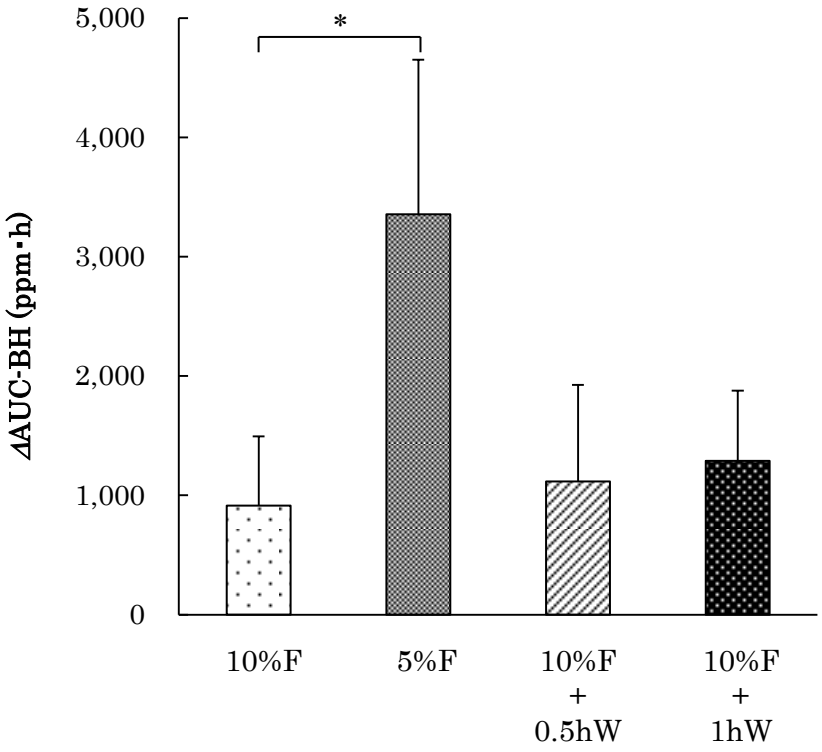


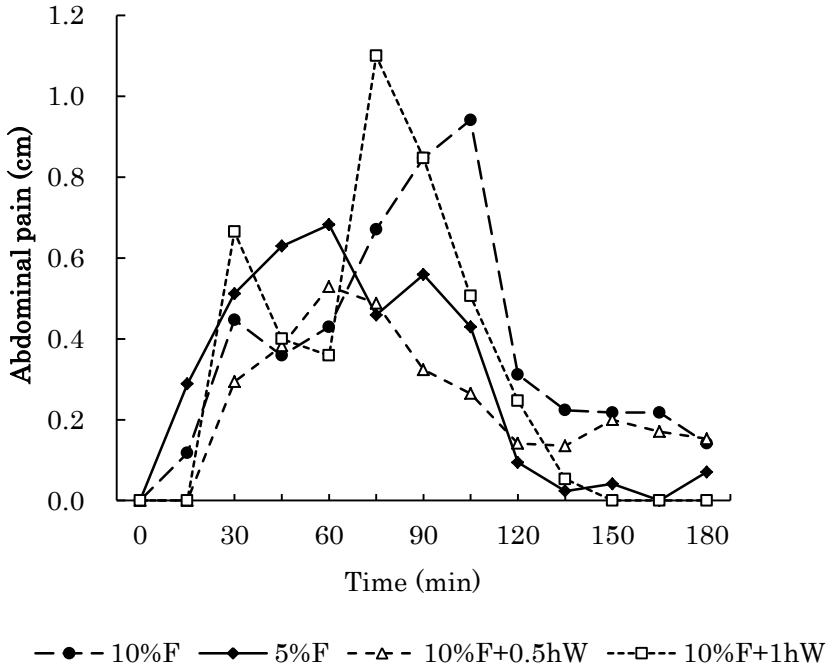
Fig. 2. ⊿BH in the four trials.
∗p<0.05 compared with the 10% F trial. Values are presented as mean.



(ppm・h)

	10%F	5%F	10%F+0.5hW	10%F+1hW
⊿AUC-BH	914.1 ± 579.6	3355.6 ± 1002.6*	1116.6 ± 809.8	1289.1 ± 589.3

Fig. 3. ⊿AUC-BH in the four trials.
Values are presented as mean± SEM.
*p<0.05 compared with the 10%F trial.



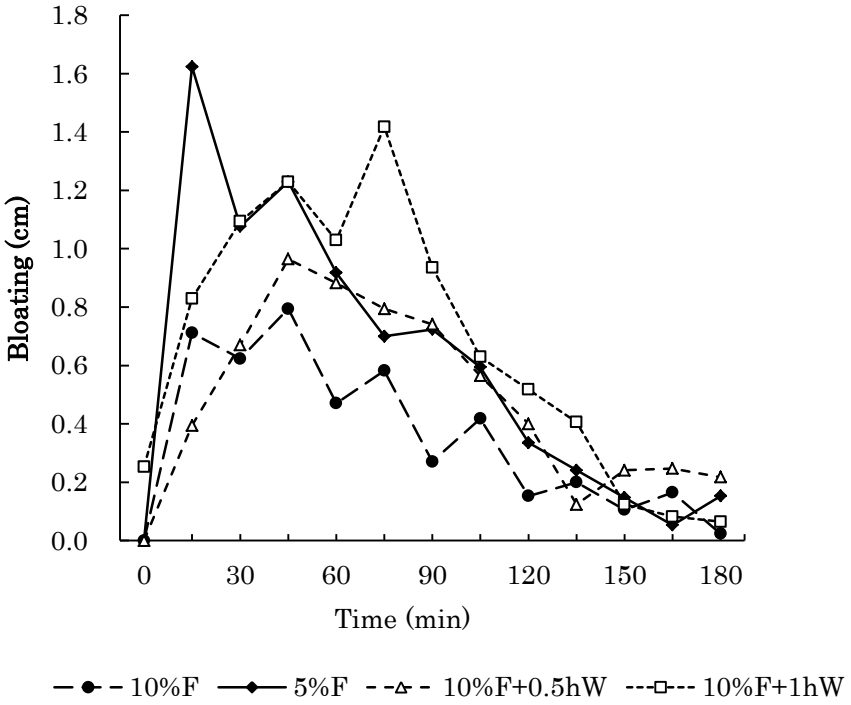
Values are presented as mean.

(cm)

Time (min)	10%F	5%F	10%F+0.5hW	10%F+1hW
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	0.12 ± 0.12	0.29 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
30	0.45 ± 0.19	0.51 ± 0.23	0.29 ± 0.17	0.66 ± 0.48
45	0.36 ± 0.21	0.63 ± 0.31	0.38 ± 0.21	0.40 ± 0.31
60	0.43 ± 0.25	0.68 ± 0.38	0.53 ± 0.32	0.36 ± 0.26
75	0.67 ± 0.40	0.46 ± 0.28	0.49 ± 0.30	1.10 ± 0.73
90	0.85 ± 0.59	0.56 ± 0.34	0.32 ± 0.16	0.85 ± 0.63
105	0.94 ± 0.62	0.43 ± 0.31	0.26 ± 0.17	0.51 ± 0.35
120	0.31 ± 0.23	0.09 ± 0.09	0.14 ± 0.14	0.25 ± 0.17
135	0.22 ± 0.22	0.02 ± 0.02	0.14 ± 0.10	0.05 ± 0.05
150	0.22 ± 0.22	0.04 ± 0.04	0.20 ± 0.15	0.00 ± 0.00
165	0.22 ± 0.20	0.00 ± 0.00	0.17 ± 0.12	0.00 ± 0.00
180	0.14 ± 0.14	0.07 ± 0.07	0.15 ± 0.15	0.00 ± 0.00

Values are presented as the means ± SEM.

Fig. 4 A. VAS of abdominal pain in the four trials.



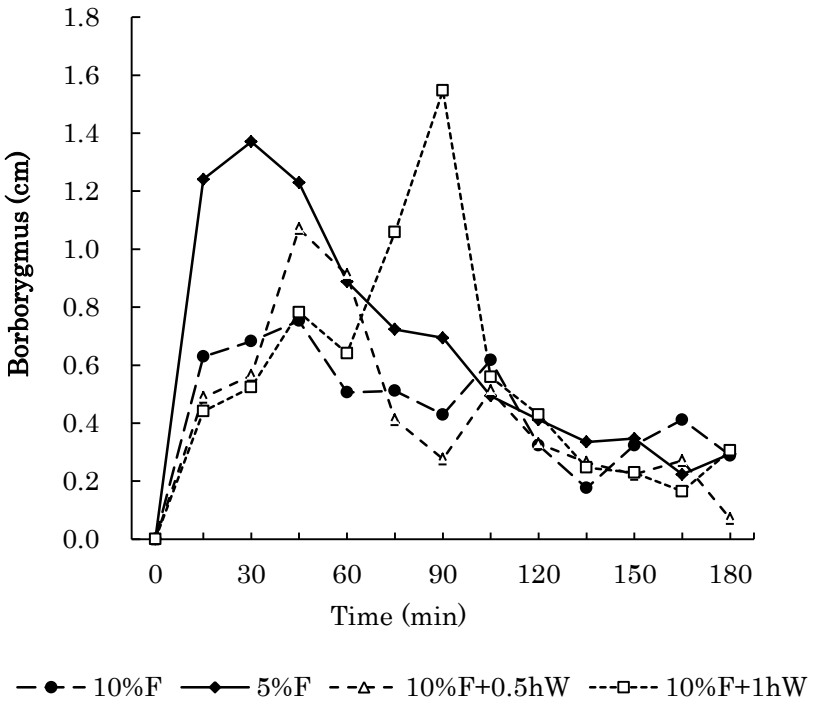
Values are presented as mean.

(cm)

Time (min)	10%F	5%F	10%F+0.5hW	10%F+1hW
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.18
15	0.71 ± 0.41	1.62 ± 0.67	0.39 ± 0.19	0.83 ± 0.47
30	0.62 ± 0.28	1.08 ± 0.46	0.67 ± 0.23	1.09 ± 0.47
45	0.79 ± 0.30	1.23 ± 0.51	0.96 ± 0.36	1.23 ± 0.53
60	0.47 ± 0.18	0.92 ± 0.41	0.88 ± 0.41	1.03 ± 0.46
75	0.58 ± 0.21	0.70 ± 0.36	0.79 ± 0.39	1.42 ± 0.69
90	0.27 ± 0.13	0.72 ± 0.30	0.74 ± 0.29	0.94 ± 0.51
105	0.42 ± 0.23	0.59 ± 0.32	0.56 ± 0.25	0.63 ± 0.39
120	0.15 ± 0.15	0.34 ± 0.15	0.40 ± 0.24	0.52 ± 0.35
135	0.20 ± 0.16	0.24 ± 0.13	0.12 ± 0.07	0.41 ± 0.33
150	0.11 ± 0.11	0.15 ± 0.08	0.24 ± 0.15	0.12 ± 0.09
165	0.16 ± 0.12	0.05 ± 0.05	0.25 ± 0.15	0.08 ± 0.07
180	0.02 ± 0.02	0.15 ± 0.13	0.22 ± 0.16	0.06 ± 0.04

Values are presented as the means ± SEM.

Fig. 4 B. VAS of bloating in the four trials.



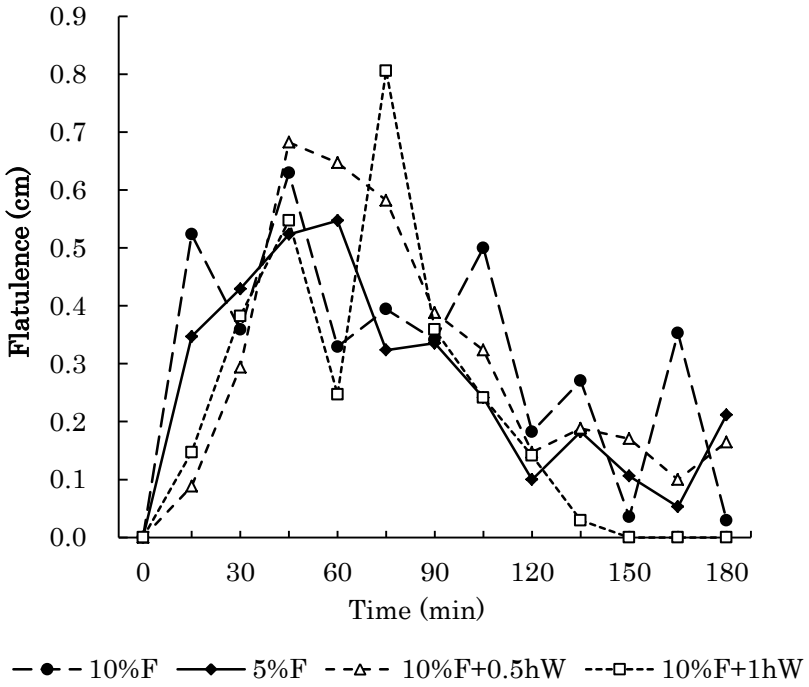
Values are presented as mean.

(cm)

Time (min)	10%F	5%F	10%F+0.5hW	10%F+1hW
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	0.63 ± 0.40	1.24 ± 0.55	0.49 ± 0.26	0.44 ± 0.30
30	0.68 ± 0.34	1.37 ± 0.44	0.56 ± 0.21	0.52 ± 0.23
45	0.75 ± 0.39	1.23 ± 0.48	1.07 ± 0.35	0.78 ± 0.29
60	0.51 ± 0.22	0.89 ± 0.33	0.91 ± 0.32	0.64 ± 0.23
75	0.51 ± 0.21	0.72 ± 0.36	0.41 ± 0.17	1.06 ± 0.50
90	0.43 ± 0.17	0.69 ± 0.30	0.28 ± 0.11	1.55 ± 0.67
105	0.62 ± 0.23	0.49 ± 0.19	0.51 ± 0.28	0.56 ± 0.28
120	0.32 ± 0.17	0.41 ± 0.17	0.33 ± 0.17	0.43 ± 0.22
135	0.18 ± 0.10	0.34 ± 0.16	0.26 ± 0.13	0.25 ± 0.14
150	0.32 ± 0.21	0.35 ± 0.16	0.22 ± 0.16	0.23 ± 0.13
165	0.41 ± 0.30	0.22 ± 0.14	0.27 ± 0.22	0.16 ± 0.11
180	0.29 ± 0.26	0.29 ± 0.14	0.07 ± 0.07	0.31 ± 0.23

Values are presented as the means ± SEM.

Fig. 4 C. VAS of borborygmus in the four trials.



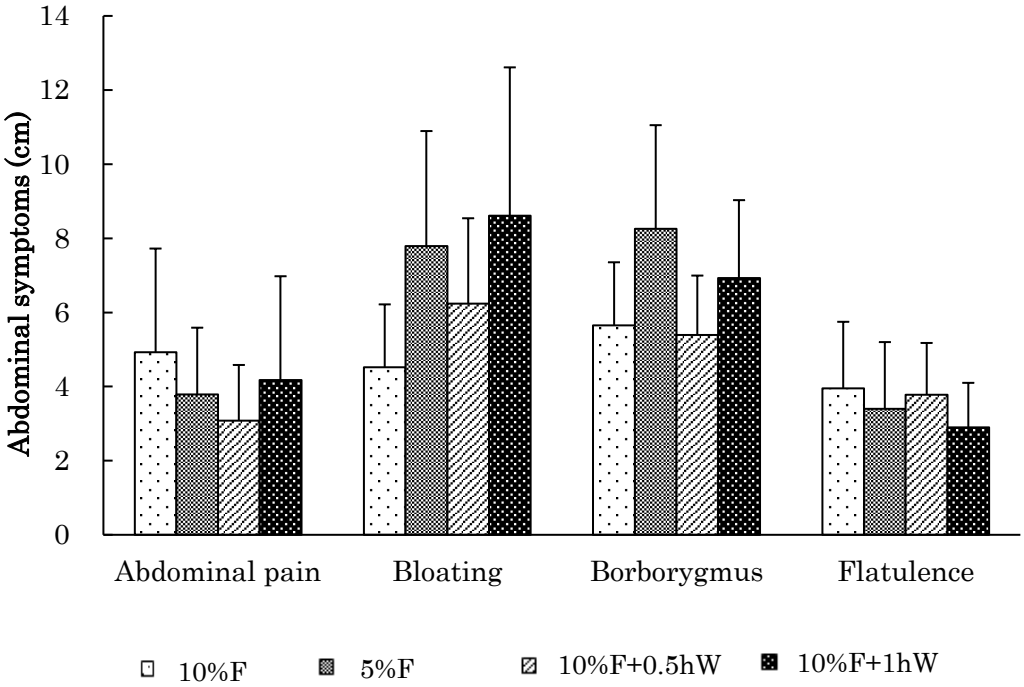
Values are presented as mean.

(cm)

Time (min)	10%F	5%F	10%F+0.5hW	10%F+1hW
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	0.52 ± 0.35	0.35 ± 0.24	0.09 ± 0.09	0.15 ± 0.08
30	0.36 ± 0.19	0.43 ± 0.24	0.29 ± 0.21	0.38 ± 0.22
45	0.63 ± 0.33	0.52 ± 0.24	0.68 ± 0.26	0.55 ± 0.24
60	0.33 ± 0.21	0.55 ± 0.24	0.65 ± 0.32	0.25 ± 0.12
75	0.39 ± 0.21	0.32 ± 0.19	0.58 ± 0.22	0.81 ± 0.48
90	0.34 ± 0.16	0.34 ± 0.22	0.39 ± 0.19	0.36 ± 0.18
105	0.50 ± 0.27	0.24 ± 0.17	0.32 ± 0.16	0.24 ± 0.16
120	0.18 ± 0.11	0.10 ± 0.07	0.15 ± 0.09	0.14 ± 0.08
135	0.27 ± 0.13	0.18 ± 0.14	0.19 ± 0.13	0.03 ± 0.02
150	0.04 ± 0.02	0.11 ± 0.07	0.17 ± 0.13	0.00 ± 0.00
165	0.35 ± 0.24	0.05 ± 0.05	0.10 ± 0.10	0.00 ± 0.00
180	0.03 ± 0.03	0.21 ± 0.16	0.16 ± 0.16	0.00 ± 0.00

Values are presented as the means ± SEM.

Fig. 4 D. VAS of flatulence in the four trials.



(cm)

	10%F	5%F	10%F+0.5hW	10%F+1hW
Abdominal pain	4.92 ± 2.82	3.79 ± 1.82	3.08 ± 1.53	4.18 ± 2.82
Bloating	4.52 ± 1.69	7.79 ± 3.14	6.24 ± 2.26	8.61 ± 4.02
Borborygmus	5.65 ± 1.72	8.25 ± 2.81	5.39 ± 1.55	6.93 ± 2.15
Flatulence	3.95 ± 1.83	3.40 ± 1.77	3.78 ± 1.40	2.90 ± 1.19

Fig. 5. Abdominal symptoms in the four trials.
The scores of each abdominal symptom were recorded at 0 minutes and every 15 minutes during a 180-minutes period and then summed (maximum score =130 cm). Values are

Table 3. The number of cases of acute diarrhea.

Trial	Number (%)
10%F	1 (5.9)
5%F	1 (5.9)
10%F+0.5hW	2 (11.8)
10%F+1hW	1 (5.9)

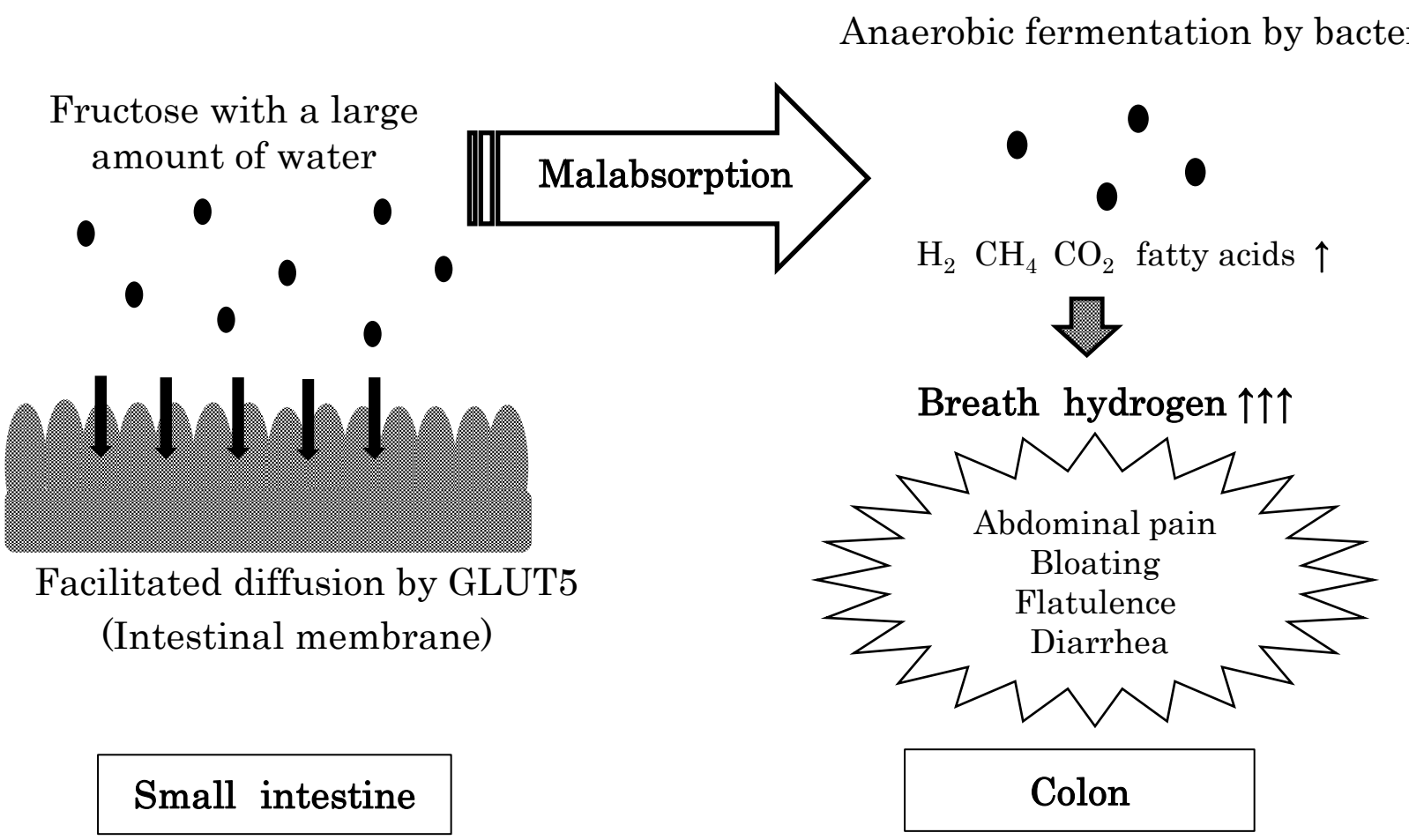


Fig. 6. Hypothesis about the mechanism of the fructose malabsorption ingested with a large amount of water.

Synthetic discussion

Fructose has the same chemical formula as glucose, namely $C_6H_{12}O_6$; however, its metabolism differs markedly from that of glucose due to its almost complete hepatic extraction and rapid hepatic conversion into glucose, glycogen, lactate, and fat. Following the ingestion of fructose, first-pass hepatic extraction is much higher than that observed in the glucose ingestion. In the liver, fructose is metabolized into glyceraldehyde and dihydroxyacetone phosphate. The ability of fructose to bypass the primary regulatory step of glycolysis, the conversion of glucose-6-phosphate to fructose 1,6-bisphosphate controlled by phosphofructokinase, is of key importance.

Furthermore, there is difference in the absorption mechanisms of fructose and glucose because the transfer of fructose into the enterocytes by facilitated diffusion may be slower than that of glucose by active transport. It is likely that gastrointestinal symptoms such as bloating, abdominal pain and diarrhea, due to the unabsorbed fructose together with a large amount of water, rapidly drifted into the colon from the small intestine and increased the production of hydrogen, methane, and fatty acids by bacterial anaerobic fermentation[1)]. When the amount of fructose is greater than that of glucose, fructose malabsorption could be induced[2, 3)].

In daily dietary habits, fructose is usually consumed with fat. For example, some beverages provided by fast-food restaurants, such as milk shakes, contain fat and high fructose corn syrup (HFCS). In this study, the ingestion of a fructose-containing beverage combined with fat cream led to a significantly higher rise with a delayed peak in the serum concentrations of TG, hTRL-TG, RLP-TG and apoB48 compared to the ingestion of a glucose-containing beverage with fat cream. These results suggest that the ingestion of fructose-containing beverage combined with fat cream delayed the intestinal absorption of dietary fat and/or the secretion of CM from the intestine, delayed the clearance of CM/CM-R from the plasma, induced a rapid lipogenesis in the liver, and enhanced the secretion of TG-richer, larger VLDL. In addition, the lower insulin excursion observed following fructose compared with glucose may also have resulted in reduced activation of LPL and led to delayed TG removal from CM and VLDL. Furthermore, the ratio of fructose to glucose in beverage may be a key factor of the metabolic disturbance.

We demonstrated that simultaneous ingestion of a high-fructose-containing beverage and fat cream caused both exogenous (derived from the intestine) and endogenous (derived from the liver) lipidemia postprandially, even in young healthy

Japanese women. It may thus have even more unfavorable effects on the health of subjects with metabolic syndrome or obesity.

At present, there are no reliable data on the upper limit of the intake of fructose and HFCS for maintaining health in Japanese individuals[4]. However, it may be advisable to avoid fructose- or HFCS-containing beverages as much as possible in daily life, even for young healthy women.

Final conclusion

Simultaneous ingestion of a high-fructose- (but not glucose-) containing beverage and fat cream caused both exogenous and endogenous lipidemia postprandially even in young healthy Japanese women. The ratio of fructose to glucose was a determinant of metabolic disturbance when sugar load was equicaloric. We also demonstrated that the fructose-contaiting beverages are liable to induce malabsorption and gastrointestinal symptoms.

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