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Original Article

Effects of continuous thiamine intake on onset and progression of type 2 diabetes in leptin-receptor deficient mice

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ABSTRACT — Aside from the COVID-19 pandemic, the obesity and diabetes pandemics have threatened global health. Patients with diabetes are more likely to experience serious complications from COVID-19; thus, preventing obesity-associated diabetes is of paramount important. Furthermore, the development of a method to prevent diabetes and elucidation of its pathology is a currently urgent issue. We previously reported that thiamine plays a key role in suppressing abnormal glycolipid metabolism in Otsuka Long-Evans Tokushima fatty (OLETF) rats, an animal model of obesity-associated diabetes. However, whether thiamine affects only OLETF rats or other animal models including a type 2 diabetes model with a different pathology requires elucidation. In this study, leptin-receptor deficiency mice were used as a model of type 2 diabetes with a different pathology to evaluate the efficacy of thiamine. The mice had free access to water containing 0.2% thiamine for 9 weeks, and the results showed that food and water consumption decreased in db/db-homo mice. Urine output, body weight gains and blood glucose levels decreased in mice that received thiamine. There were 5 mice and 1 mouse with a fasting glucose level of ≥ 300 mg/dL in the db/db-homo control group (n = 10) and db/db-homo thiamine group (n = 10), respectively, suggesting that thiamine intake may suppress an increase in blood glucose levels. The results of the present study suggest that demand for thiamine may exceed the normal range in *in vivo* mouse models of diabetes and continuous thiamine intake affects diabetes onset and progression.

Key words: Thiamine, Diabetic db/db mice, Obesity, Type 2 diabetes, Body weight gains, Blood glucose level

INTRODUCTION

Owing to global changes to lifestyle habits and social environments, the number of patients with diabetes has rapidly increased (Ng *et al*., 2014; WHO, 2016, 2017; Afshin *et al*., 2017; Kohda, 2018; IDF, 2021). The diabetes pandemic has become a major health concern worldwide (IDF, 2021). Diabetes is associated with several complications and symptomatic therapy is provided for each complication. However, few effective method, including drugs, to prevent diabetes has been established yet.

Thiamine, which is involved in glucose metabolism, acts as a coenzyme of dehydrogenase and transketolase. Dehydrogenase catalyzes oxidative decarboxylation of α-ketoglutaric acids and transketolase transfers keto groups. Thiamine is reported to be effective in preventing the progression of diabetic complications (Brownlee, 2001; Babaei-Jadidi *et al*., 2003, 2004; Hammes *et al*., 2003; Ceylan-Isik *et al*., 2006). Thiamine-responsive megaloblastic anemia (TRMA) is a thiamine deficiency that contributes to the occurrence and exacerbation of type 2 diabetes (Abboud *et al*., 1985;

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Borgna-Pignatt *et al*., 1989; Neufeld *et al*., 2001; Alzahrani *et al*., 2006). TRMA is an autosomal recessive genetic disorder caused by mutations in the *SLC19A2*, a gene encoding the thiamine transporter (Abboud *et al*., 1985; Borgna-Pignatti *et al*., 1989; Neufeld *et al*., 2001; Alzahrani *et al*., 2006). TRMA is a rare, single-gene disorder characterized by anemia, hearing loss, and type 2 diabetes caused by impaired active absorption of thiamine into cells (Abboud *et al*., 1985; Borgna-Pignatti *et al*., 1989; Neufeld *et al*., 2001; Alzahrani *et al*., 2006). A meta-analysis reported that *SLC19A2* pleomorphism in humans is involved in the onset of type 2 diabetes (Hanson *et al*., 1998; Elbein *et al*., 1999).

Although high thiamine doses suppress the progression of diabetic complications, methods to prevent diabetes have not been established thus far. In our previous study, we evaluated the treatment method to prevent obesity and overall abnormal metabolism using Otsuka Long-Evans Tokushima fatty (OLETF) rats as a model of diabetes (Tanaka *et al*., 2010; Kohda *et al*., 2012, 2017; Kohda and Matsumura, 2019; Kohda, 2020). OLETF rats lack cholecystokinin A receptors and consequently experience hyperphagia (Kawano *et al*., 1991, 1992; Funakoshi *et al*., 1994). This leads to the onset of obesity, type 2 diabetes, and other metabolic disorders. Currently, owing to such characteristics, OLETF rats are a widely used animal model of type 2 diabetes and metabolic syndrome (Kawano *et al*., 1991, 1992; Funakoshi *et al*., 1994). A 55-week continuous study reported numerous positive outcomes of administering water containing thiamine including decrease in body weight gain mainly via visceral fat loss, improvement in results of laboratory tests, suppression of adipocyte enlargement, decreases in fat accumulation and fatty acid degeneration in the liver and pancreas, and improvement in cardiac function by decreasing fat accumulation in the heart (Tanaka *et al*., 2010; Kohda *et al*., 2012). Taken together, we reported the efficacy of thiamine to suppress obesity and metabolic syndrome secondary to hyperphagia in OLETF rats (Tanaka *et al*., 2010; Kohda *et al*., 2012).

In the present study, we used db/db-homo mice of type 2 diabetes lacking leptin receptors to evaluate whether thiamine affects only OLETF rats lacking cholecystokinin receptors or other animal models of type 2 diabetes with a different pathology. Leptin receptors are single-transmembrane proteins that belong to the gp130 family of the cytokine receptor superfamily; these receptors are mainly located in the hypothalamus (Febbraio, 2007). db/db mice lack leptin receptors, they consequently develop hyperphagia and present with fat accumulation, leading to obesity (Koya *et al*., 2000; Wang *et al*., 2014). Obesity symptoms start to develop at the age of 3–4 weeks and blood glucose levels at 4–8 weeks (Koya *et al*., 2000; Wang *et al*., 2014). Furthermore, symptoms suggestive of exhaustion of βcells of the pancreatic islet are noted, which include hyperphagia, polydipsia, and polyuria. Owing to such characteristics, db/db mice are a useful animal model of type 2 diabetes (Wang *et al*., 2014). In this study, we used the db/db-homo mice as animal models of spontaneous type 2 diabetes and the db/db-hetero mice with a heterozygous genotype, to evaluate the effect of continuous thiamine intake on obesity and diabetes.

MATERIALS AND METHODS

Animals and experimental design

This study was performed after obtaining approval from the Experimental Animal Research Committee of the Osaka Medical and Pharmaceutical University. Five-week old male db/db-homo mice (BKS.Cg-m+/+Lepr^{db}/J, n = 20; Jackson Laboratory Japan, Inc., Kanagawa, Japan) and db/db-hetero mice (n = 20; Jackson Laboratory Japan, Inc., Kanagawa, Japan) were obtained and kept in the animal room under a 12-hr light-dark cycle with constant temperature (24°C ± 1°C) and humidity (55% ± 5%). They had free access to conventional pellets (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The mice were divided into the following 4 groups in 8 cages (i.e., 10 mice for each group [2 cages for each group]) while ensuring that the mean body weight (g) of the mice in each group was similar: I, db/db-homo control group; II, db/db-homo thiamine group; III, db/db-hetero control group; and IV, db/db-hetero thiamine group. Ad libitum access was provided for tap water alone and tap water containing thiamine hydrochloride 2 g/L (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) to the control and thiamine groups, respectively. During a 9-week observation period, body weight, water and food consumption were measured once a week.

The mice were transferred to metabolic cages for a 16-hr urine collection. During the urine collection, 5 mice were kept in a metabolic cage to collect pooled urine samples. Urine collection was performed under fasting conditions and urine volume was measured. On the experiment week 9, after mice were fasted for 16 hr, blood was partially collected from the tail vein using a razor without anesthesia. Approximately 80 μL blood was collected with a heparin sodium-coated capillary tube (Thermo Fisher Scientific Inc., MA, USA).

At necropsy, which was performed following completion of the partial blood collection, whole blood was collected from the abdominal aorta under anesthesia.

The following samples were collected: heart, liver, kidneys, pancreas, epididymal fat, retroperitoneal fat, and brown fat on the back. The right hindlimb was excised and placed in hot water for boiling with the aim of clearly shaving muscle tissues around the tibia. The tibia length was measured with a digital caliper (AS ONE Corp., Osaka, Japan).

The collected blood was centrifuged at 3500 rpm, 4°C for 15 min to obtain the plasma. Blood chemistry was performed using the collected plasma sample. Fuji Dri-Chem 3500 V (FUJIFILM Corp., Tokyo, Japan) was used as a measurement system. Blood glucose levels and triglycerides were measured using the glucose oxidase method and lipoprotein lipase method, respectively. The collected plasma was diluted if a value was beyond the detection limit (glucose levels ≥ 600 mg/dL, triglycerides ≥ 500 mg/dL).

Statistical analyses

Data are expressed as the means ± S.E. Statistical analyses of the data from multiple groups were performed by analysis of variance followed by Bonferroni tests. All statistical analyses were performed using Pharmaco Basic software (Scientist Press Co., Ltd., Tokyo, Japan). A p-value < 0.05 was considered to indicate statistically significant results.

RESULTS

Body weight changes as an indicator for obesity

A remarkable increase in body weight was noted in the db/db-homo group, a spontaneous type 2 diabetic animal model, compared with the db/db-hetero group, a mouse with a heterozygous genotype (Fig. 1A). In cases when the body weight at baseline was used as the standard, changes in body weight were lower in the db/db-homo thiamine group than in the db/db-homo control group during weeks 2–8 (Fig. 1B). Week 8 onward, db/db-homo control group started to show slight decreases in body weight, following which similar body weight changes were noted between the db/db-homo control group and db/db-homo thiamine group (Fig. 1A and 1B). No significant difference was noted in body weight changes between the db/db-hetero control and db/db-hetero thiamine groups from baseline to the experiment week 9.

Changes in food and water consumption

A remarkable increase in food consumption was noted in the db/db-homo group compared with the db/db-hetero group (Fig. 2A). No major differences were noted in terms of changes in food consumption between the db/db-homo control group and db/db-homo thiamine group from baseline to week 3 (Fig. 2A). However, food consumption started to decrease in the db/db-homo thiamine group week 4 onward, and remained lower than that in

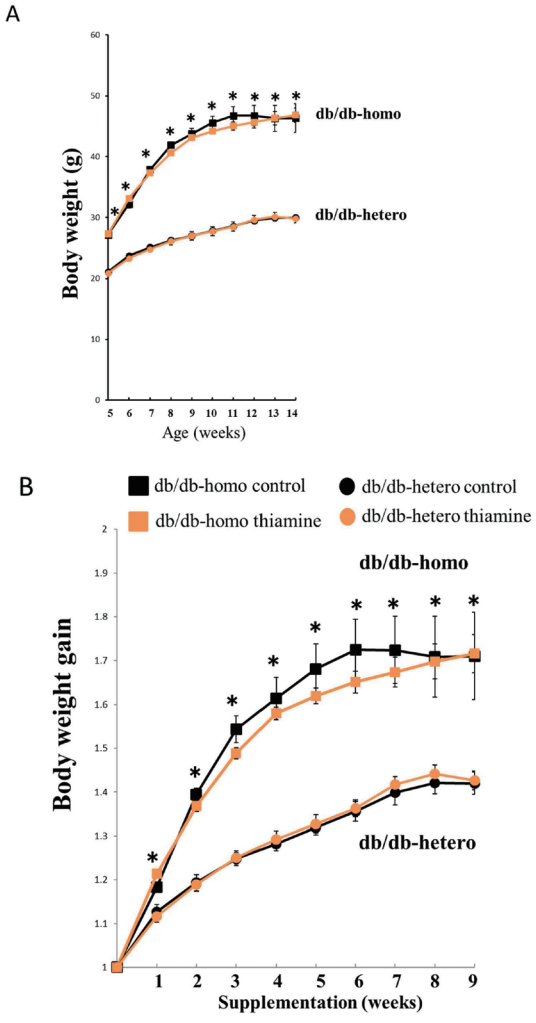

Fig. 1. Body weight (A) and body weight gain (B) changes from 5 weeks of age and at week 9 of thiamine supplementation in db/db-homo and db/db-hetero mice. Effect of thiamine on body weight change in db/db-homo and db/db-hetero mice with 2 g thiamine/L of drinking water for 9 weeks. db/db-homo and db/db-hetero mice were randomly divided into the following groups: unsupplemented control diabetic group (db/db-homo control, n = 10), thiamine-supplemented diabetic group (db/db-homo thiamine, n = 10), unsupplemented normal group (db/db-hetero control, n = 10) and thiamine-supplemented normal group (db/db-hetero thiamine, n = 10). Each value represents the mean ± S.E. values. *p < 0.01 compared to the normal group (db/db-hetero control).

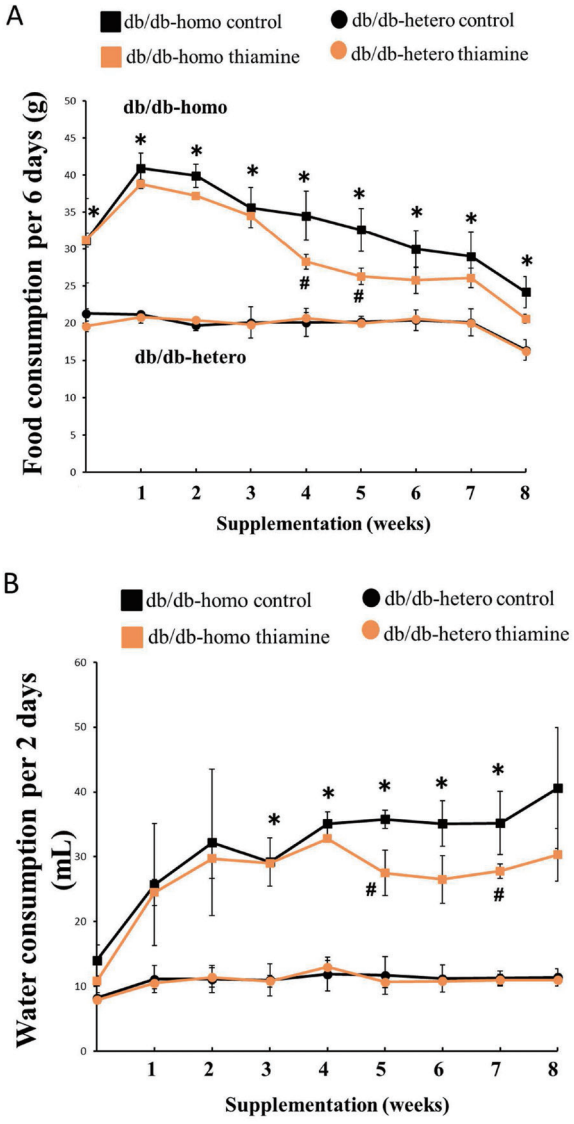

Fig. 2. Average food consumption per 6 days (A) and water consumption per 2 days (B) from 5 weeks of age and at week 9 of thiamine supplementation in db/db-homo and db/db-hetero mice. Effects of thiamine on food and water consumption in db/db-homo and db/db-hetero mice with 2 g thiamine/L of drinking water for 9 weeks. db/db-homo and db/db-hetero mice were randomly divided into the following groups: unsupplemented control diabetic group (db/db-homo control, n = 10), thiamine-supplemented diabetic group (db/db-homo thiamine, n = 10), unsupplemented normal group (db/db-hetero control, n = 10) and thiamine-supplemented normal group (db/db-hetero thiamine, n = 10). Each value represents the mean ± S.E. values. *$p < 0.01$ compared to the normal group (db/db-hetero control). #$p < 0.05$ compared to the diabetic group (db/db-homo control).

the control group to the experiment week 9 (Fig. 2A). We did not observe any significant difference in changes in food consumption between the db/db-hetero control and db/db-hetero thiamine groups from baseline to the experiment week 8 (Fig. 2A).

A remarkable increase in water consumption was noted in the db/db-homo group compared with the db/db-hetero group (Fig. 2B). No significant difference in changes in water consumption between the db/db-homo control and db/db-homo thiamine groups from baseline to week 4 (Fig. 2B). However, water consumption started decreasing in the db/db-homo thiamine group from week 5, and remained lower than that in the control groups till the last experiment day (Fig. 2B). We did not observe any significant difference in changes in water consumption between the db/db-hetero control and db/db-hetero thiamine groups from baseline to the last experiment day (Fig. 2B).

Changes in urine output, organ weight and tibia length

Remarkable increase in urinary volume, epididymal fat, retroperitoneal fat, brown fat, and liver weight were noted in the diabetic db/db-homo group, compared with the normal db/db-hetero group (Fig. 3A, 3B, 3C, 3D, 3G). The db/db-homo thiamine group tended to have a lower urine output than the db/db-homo control group, and no significant difference was noted between the db/db-hetero control and db/db-hetero thiamine groups (Fig. 3A). No significant difference was observed in terms of the mean weight of the heart, liver, kidneys, epididymal fat, retroperitoneal fat, and tibia length between the db/db-homo control and db/db-homo thiamine groups (Fig. 3B, 3C, 3E, 3F, 3G, 3H, 3I). The mean brown fat weight tended to be lower in the db/db-homo control group than in the db/db-homo thiamine group (Fig. 3D).

Effects of thiamine on blood glucose and triglyceride levels in diabetic db/db mice

The db/db-homo groups (i.e., type 2 diabetes model) showed a notably higher increase in blood glucose levels than the db/db-hetero groups (i.e., heterozygous mice) (Fig. 4A). After the mice were fasted for 16 hr, 5 mice in the db/db-homo control group (n = 10) and 1 mouse in the db/db-homo thiamine group (n = 10) had a blood glucose level of ≥ 300 mg/dL (Fig. 4B). The mean blood glucose level was significantly lower in the db/db-homo thiamine group than in the db/db-homo control group (Fig. 4A). An increase in triglycerides tended to be higher in the db/db-homo groups than in the db/db-hetero groups (Fig. 4C). The triglycerides showed no difference between the db/db-homo control and db/db-homo thia-

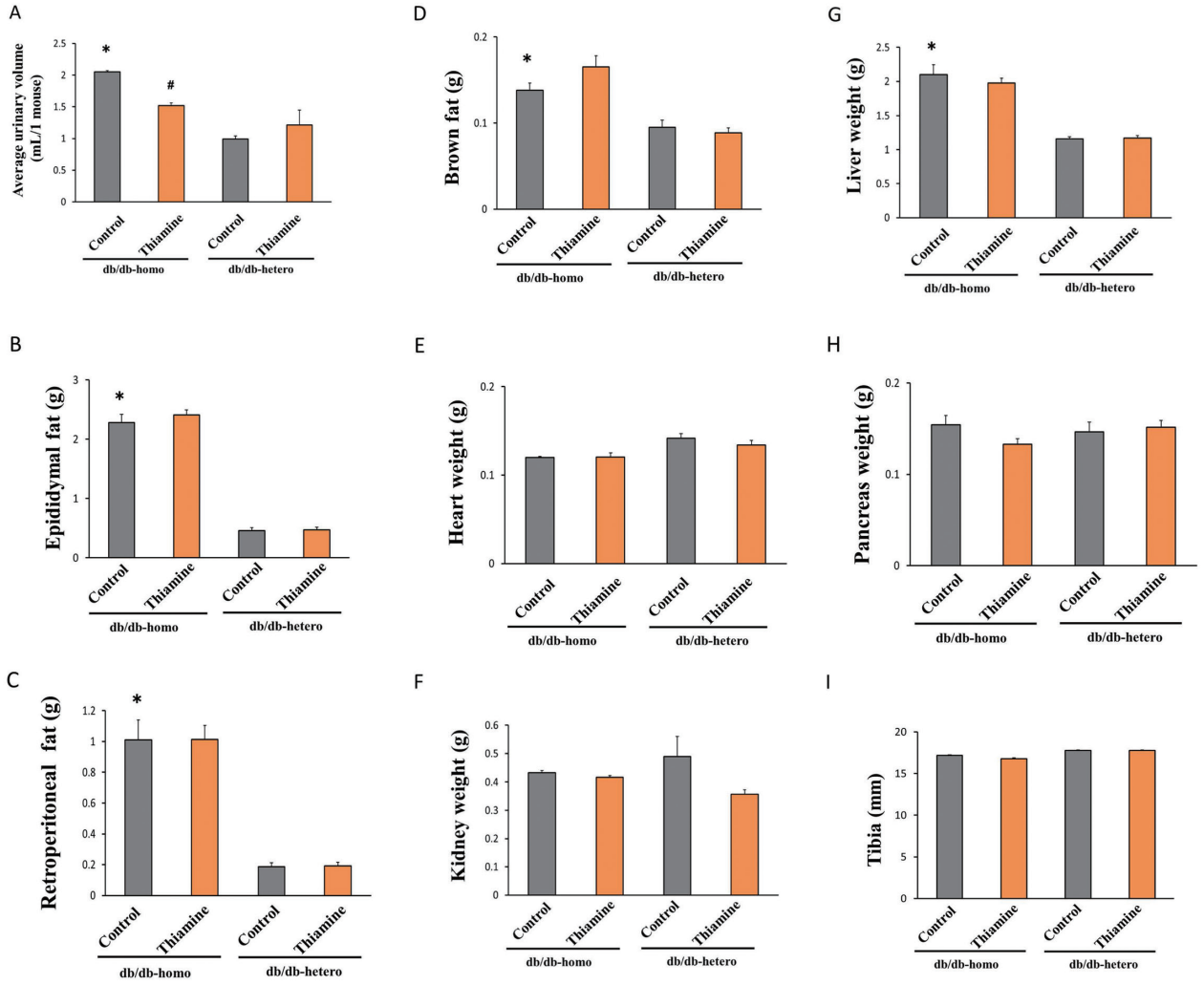

Fig. 3. Level of urinary volume (A), organ weight (B-H) and tibia length (I) at week 9 of thiamine supplementation in db/db-homo and db/db-hetero mice. Effects of thiamine on urinary volume, organ weight, and tibia length in db/db-homo and db/db-hetero mice with 2 g thiamine/L of drinking water for 9 weeks. db/db-homo and db/db-hetero mice were randomly divided into the following groups: unsupplemented control diabetic group (db/db-homo control, n = 10), thiamine-supplemented diabetic group (db/db-homo thiamine, n = 10), unsupplemented normal group (db/db-hetero control, n = 10) and thiamine-supplemented normal group (db/db-hetero thiamine, n = 10). Each value represents the mean ± S.E. values. *p < 0.05 compared to the normal group (db/db-hetero control).

mine groups (Fig. 4C). No significant difference was noted in results of blood chemistry between the db/db-hetero control and db/db-hetero thiamine groups (Fig. 4A, 4B and 4C).

DISCUSSION

This study showed that intake of thiamine 0.2% (w/v) for 9 weeks reduced the food and water consumption of the db/db-homo mice (i.e., animal model of spontaneous type 2 diabetes). Urine collected on the last experiment day tended to be lower in the thiamine groups than in the other groups. Five mice in the db/db-homo control group (n = 10) and 1 mouse in the db/db-homo thiamine group (n = 10) had a fasting blood glucose level of ≥ 300 mg/dL. This suggested that thiamine intake may suppress an increase in blood glucose levels. We compared tissue weight between the thiamine and control

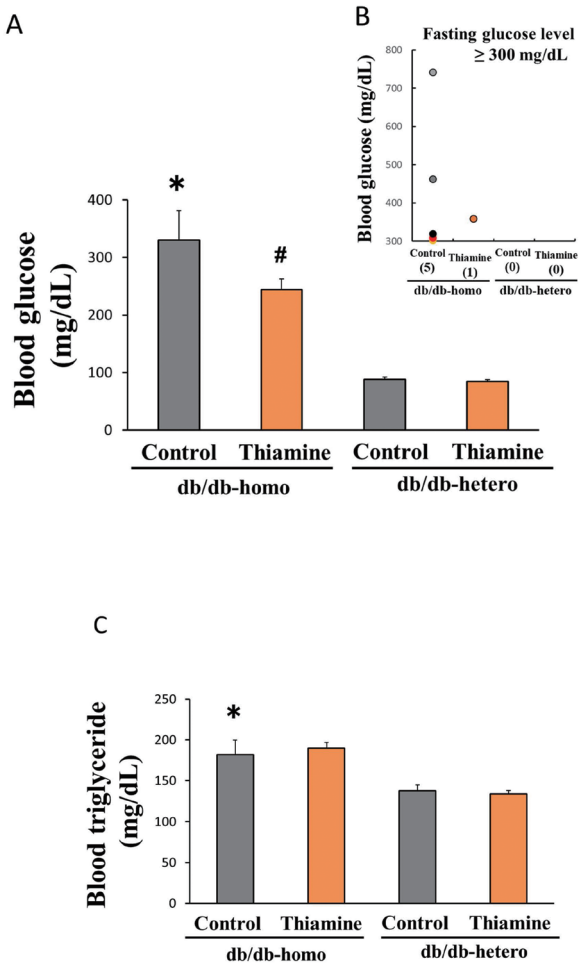

Fig. 4. Blood glucose (A), fasting glucose level of ≥ 300 mg/dL (B) and triglyceride (C) at week 9 of thiamine supplementation in db/db-homo and db/db-hetero mice. Effects of thiamine on blood glucose and triglyceride levels in db/db-homo and db/db-hetero mice with 2 g thiamine/L of drinking water for 9 weeks. db/db-homo and db/db-hetero mice were randomly divided into the following groups: unsupplemented control diabetic group (db/db-homo control, n = 10), thiamine-supplemented diabetic group (db/db-homo thiamine, n = 10), unsupplemented normal group (db/db-hetero control, n = 10) and thiamine-supplemented normal group (db/db-hetero thiamine, n = 10). Each value represents the mean ± S.E. values. *$p < 0.01$ compared to the normal group (db/db-hetero control). $^{\#}p < 0.05$ compared to the diabetic group (db/db-homo control).

groups. We presumed that, in the control groups, fatty liver, enlargement of the pancreas caused by the excessive production of insulin, and atrophy of brown fat, changes into white fat, had progressed. In contrast, progression of these symptoms tended to be suppressed in the thiamine groups. However, to establish reliable conclusions, discussion based on histopathology is essential; this warrants further study in the future. Such different findings were observed only in db/db-homo mice. In the db/db-hetero mice used as normal mice, there were no differences in all parameters including food and water consumption and blood glucose levels between the thiamine and control groups. Whether the abovementioned differences observed between db/db-homo mice and db/db-hetero mice resulted from improvement in hyperphagia through leptin receptor mutation or resulted from other mechanisms unrelated to food consumption remained unclear; Thus, further investigation is required.

The body weight of male db/db-homo mice (5 weeks of age) obtained from a breeder was approximately 30% higher than that of the db/db-hetero mice (db/db-homo: 27.3 g vs. db/db-hetero: 21.0 g). Obesity might play a role in diabetic condition of the db/db-homo mice. This study showed the effect of thiamine on a mouse model of severe diabetes. Thiamine is water-soluble and transferred to infants through breast milk. We plan to conduct a study to examine the effect of thiamine on the prevention of diabetes through the administration of thiamine to db/db mice during pregnancy, lactation, and weaning phases.

In this study, db/db-homo control group started to show slight decreases in body weight from week 8 onward. However, such body weight changes were not observed in the db/db-homo thiamine group. Pancreatic β-cells are damaged in patients with advanced type 2 diabetes, leading to fulminant type 1 diabetes (Nakamura *et al*., 2008; Nishida *et al*., 2014; Kim *et al*., 2017). Week 8 onward, diabetic db/db mice started to show slight decreases in body weight, and 2 mice had a high fasting blood glucose level (> 400 mg/dL), thus possibly suggesting that these mice were transitioning from type 2 to type 1 diabetes. Thiamine intake may have mitigated destruction of β cells of the pancreas (Tanaka *et al*., 2010; Kohda *et al*., 2012).

Leptin, which is produced in lipocytes in the body, transmits the rough amount of body fat to the brain in order to regulate food intake and metabolism (MacDougald *et al*., 1995; Campfield *et al*., 1996; Hamann and Matthaei, 1996; Girard, 1997; Houseknecht *et al*., 1998a, 1998b). Leptin inhibits neuropeptide Y and agouti-related protein-expressing neurons to increase alpha-melanocyte stimulating hormone activity (Meinders, *et al*., 1996; Schwartz *et al*., 1996; Houseknecht *et al*., 1998a, 1998b; Margetic *et al*., 2002; Mark *et al*., 2009). OLETF rats, which lack cholecysto-

kinin receptors, experience obesity and other obese-related conditions secondary to overeating. Cholecystokinin is a digestive hormone. Its secretion from I-cells in the jejunum and duodenum is promoted by peptides, amino acids, and fatty acids in the duodenum. This activates phospholipase C on pancreatic gland cell and increases inositol trisphosphate. As a result, cholecystokinin promotes secretion of pancreatic enzymes and transmits a satiety signal to the brain (Funakoshi *et al*., 1995; Takiguchi *et al*., 1997; Smith, 2006).

Both leptin-receptor deficiency and cholecystokinin-receptor deficiency are associated with overeating; however, they involve different mechanisms of secretion. Leptin transmits the rough amount of body fat to the brain, while cholecystokinin transmits the amount of food intake to the brain. We previously reported that thiamine improved obesity and obesity-related abnormal glycolipid metabolism in OLETF rats (Tanaka *et al*., 2010; Kohda *et al*., 2012). However, the present study showed that such effects on db/db mice were limited. Differences in secretion mechanism may partially explain the differences in the effect of thiamine.

Although the world has entered the post-COVID period, there are no signs that the obesity and diabetes pandemic will come under control. Patients with diabetes are more likely to have serious complications from COVID-19. We believe that prevention of diabetes secondary to obesity is important in the current eras of the diabetes pandemic.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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