The Japanese Society of Toxicology

Fundamental Toxicological Sciences
URL : http://www.fundtoxicolsci.org/index_e.html

Original Article

Thiamine supplementation modulates oxidative stress by inhibiting hepatic adenosine diphosphate (ADP)-ribosylation in obese diabetic rats

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(Received December 17, 2018; Accepted December 30, 2018)

ABSTRACT — Diabetic hyperglycemia is typically accompanied by various protein modifications, indicating hyperglycemic glucotoxicity. Overactivation of poly [adenosine diphosphate (ADP)-ribose] polymerase 1 (PARP-1) has been implicated in the pathogenesis of oxidative stress-related diseases including diabetes and its complications. Furthermore, obesity and diabetes are known to be associated with a substantial risk of chronic liver disease. We have previously reported that thiamine supplementation prevented obesity and diabetes-related liver disease. As a step forward, in the present study, we focus on hepatic ADP-ribosylation that reflects PARP-1 activation and an increased oxidative stress condition. Otsuka Long-Evans Tokushima Fatty (OLETF) rats were randomly divided into the following groups: thiamine-supplemented and unsupplemented control groups. The thiamine-supplemented group received 2 g of thiamine/L of drinking water for 33 weeks. ADP-ribosylated protein expression was analyzed in the livers of OLETF rats using Western blotting. Moreover, the fasting blood glucose level was measured in these rats. The obese diabetic OLETF rats exhibited high ADP-ribosylated protein expression in the liver. Interestingly, hepatic ADP-ribosylated protein expression and fasting blood glucose levels were lower in the thiamine-supplemented OLETF group than in the control OLETF group. These results suggest that thiamine supplementation attenuates oxidative stress by inhibiting hepatic ADP-ribosylation in OLETF rats. The beneficial effect of high-dose thiamine on oxidative stress-related diseases could be attributed to its inhibitory effect on PARP-1 activation, in addition to its role as a coenzyme. Furthermore, we found that thiamine supplementation prevented fasting hyperglycemia, suggesting that high-dose thiamine modifies the hepatic glucose metabolism and obesity-induced hepatic insulin resistance.

Key words: Glucotoxicity, Oxidative stress, Hepatic ADP-ribosylation, Obese diabetes, Thiamine supplementation, Fasting hyperglycemia

INTRODUCTION

Chronic hyperglycemia causes diabetes and associated complications (Poitout and Robertson, 2002; Kaiser *et al*., 2003; Roseman, 2005; Del Prato, 2009; Weir *et al*., 2009). Diabetic hyperglycemia is typically accompanied by various protein modifications, indicating hyperglycemic glucotoxicity (Wolff *et al*., 1991; Dunlop, 2000; Chung *et al*., 2003; Beyer and Weihrauch, 2012; Vlassara and Striker, 2013; Yan, 2014; Kohda *et al*., 2008, 2009, 2012a). Post-translational protein modifications could be attributed to oxidative stress induced by redox imbalance in cases of

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diabetes (Yan, 2014). Oxidative stress occurs when the level of released reactive oxygen species (ROS) exceeds the antioxidant capacity (Waddington *et al*., 2000; Chapple and Matthews, 2007). Several studies have established an association between ROS overproduction and hyperglycemic glucotoxicity (Evans *et al*., 2003). Overactivation of poly [adenosine diphosphate (ADP)-ribose] polymerase 1 (PARP-1) has been implicated in the pathogenesis of oxidative stress-related diseases (Pacher and Szabó, 2005; Szabó, 2005; Luo and Kraus, 2012). In target organs under oxidative stress conditions, proteins are susceptible to diabetic ROS-induced protein modifications, such as protein ADP-ribosylation (Masutani *et al*., 1999; Pieper *et al*., 1999; Virág and Szabó, 2002; Daniels *et al*., 2015).

Diabetes is known to be associated with a substantial risk of chronic liver disease (Moscatiello *et al*., 2007; Arrese, 2010; Porepa *et al*., 2010). Diabetes itself can directly cause cirrhosis, potentially because of the progression of nonalcoholic fatty liver disease (NAFLD) (Cusi, 2009). NAFLD is a general term for all fatty liver diseases occurring in the absence of a drinking history associated with alcoholic hepatopathy (Adams and Angulo, 2005; Porepa *et al*., 2010). NAFLD is a very common disorder among obese patients with diabetes (Adams, 2007). The incidences of obesity and metabolic syndrome have increased globally, and NAFLD has become the most common liver disease, accelerating the need for countermeasures (Lazo and Clark, 2008; Argo and Caldwell, 2009; Bellentani and Marino, 2009; Bellentani *et al*., 2010).

Thiamine (vitamin B1), which plays an important role in glucose metabolism, can prevent diabetic complications, including those in obesity (Babaei-Jadidi *et al*., 2003, 2004; Hammes *et al*., 2003; Thornalley, 2005; Beltramo *et al*., 2008). We previously found that thiamine supplementation could influence metabolic abnormalities, such as progressive obesity and metabolic disorders similar to human metabolic syndrome, in polyphagia-induced Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Tanaka *et al*., 2010; Kohda *et al*., 2012b). In OLETF rats, thiamine supplementation averted obesity, mainly through a reduction in visceral adiposity, and prevented metabolic disorders (Tanaka *et al*., 2010; Kohda *et al*., 2012b). The transcript expression levels of genes, which were assessed using gene microarrays, indicated a difference in hepatic expression with high-dose thiamine supplementation (Tanaka *et al*., 2010; Kohda *et al*., 2012b, 2017). Several of these genes were found to participate in carbohydrate metabolism, lipid metabolism, vascular physiology, and carcinogenesis (Tanaka *et al*., 2010; Kohda *et al*., 2012b, 2017). Although corroboration is necessary, our previous findings indicate that thiamine has the potential to prevent obesity and metabolic disorders in OLETF rats (Tanaka *et al*., 2010; Kohda *et al*., 2012b, 2017).

In our previous study, thiamine supplementation ameliorated body weight gain by suppressing visceral fat generation in OLETF rats (Tanaka *et al*., 2010; Kohda *et al*., 2012b). Additionally, hepatic triglyceride accumulation was significantly reduced with high-dose thiamine supplementation (Tanaka *et al*., 2010; Kohda *et al*., 2012b). Moreover, we noted improvements in biochemical findings, degree of fatty liver, and obesity-related hepatic pathology and dysfunction (Tanaka *et al*., 2010; Kohda *et al*., 2012b, 2017). As a step forward, in the present study, we focused on protein modification, i.e., hepatic ADP-ribosylation that reflects PARP-1 activation and an increased oxidative stress condition.

MATERIALS AND METHODS

Chemicals

Thiamine hydrochloride was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan). A glucose pilot meter and blood glucose test strips (Aventir Biotech, Carlsbad, CA, USA) were used for blood glucose testing. Anti-poly (ADP-ribose) polymer antibody was purchased from Tulip Bio Labs (Lansdale, PA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Saline solution was supplied by Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Mammalian tissue lysis and extraction reagents were purchased from Sigma (St Louis, MO, USA). Protease inhibitor cocktail and blocking solution were supplied by Nacalai Tesque (Kyoto, Japan). All other chemicals used were of the highest purity available from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and experimental design

We previously found that high-dose thiamine prevents obesity and diabetic complications in OLETF rats that exhibit progressive obesity and metabolic disorders (Tanaka *et al*., 2010; Kohda *et al*., 2012b, 2017). In this study, we additionally assessed Long-Evans Tokushima Otsuka (LETO) rats, which are the nondiabetic/lean counterparts of OLETF rats. Five-week-old male OLETF and LETO rats (Japan SLC, Inc., Shizuoka, Japan) were randomly divided into the following groups: unsupplemented control OLETF group (n = 6), thiamine-supplemented OLETF group (n = 6), unsupplemented control LETO group (n = 3), and thiamine-supplemented LETO group

(n = 3). The thiamine-supplemented group received 2 g of thiamine/L of drinking water for 33 weeks. The rats were housed in standard cages (2 rats/cage) with free access to water and standard rodent chow, unless stated otherwise. The selected thiamine dose was consistent with the dose used in a previous study on OLETF rats (Tanaka *et al*., 2010; Kohda *et al*., 2012b). The cages were placed in temperature-controlled animal quarters (21°C) under a 12 hr (0700-1900 hr):12 hr (1900-0700 hr) light:dark cycle, which was maintained throughout the experimental period, and the cages were changed at the beginning of each week. The body weights of the rats were measured weekly between 1000 and 1100 hr throughout the experimental period. The rats received ear piercings indicating their individual numbers for long-term breeding observation experiments from 5 weeks to 38 weeks of age (Fig. 1A). The experimental protocols and animal care methods were approved by the Experimental Animal Research Committee of Osaka University of Pharmaceutical Sciences.

Measurement of casual and fasting blood glucose levels

Glucose levels were measured in blood collected from the tail vein of the rats, using a blood glucose test strip (Aventir Biotech). The casual blood glucose level was measured every 2 weeks throughout the experimental period. When blood was collected after a 24-hr fast, the glucose level was considered as the fasting blood glucose level.

Blood and tissue sampling

The OLETF and LETO rats were sacrificed at 38 weeks of age. The rats were anesthetized with 50 mg/kg pentobarbital. Blood samples were collected from the ventral aorta in heparin tubes. Furthermore, plasma was separated from whole blood by centrifugation using a refrigerated bench-top centrifuge (Kubota Corp., Tokyo, Japan), and plasma aliquots were stored at −80°C until further analysis. Plasma triglyceride and cholesterol levels were measured using triglyceride test and total cholesterol test kits, respectively. The rats were exsanguinated, and liver, epididymal fat, and retroperitoneal fat samples were collected, rinsed in ice-cold saline, briefly blotted with paper, and weighed.

Preparation of protein extraction samples from rat livers

Immediately following liver extraction and weighing, liver tissues were immersed and rinsed in ice-cold saline. Then, the tissues were minced and 100 mg of the minced tissues was homogenized at 4°C in 900 µL of tissue lysis and extraction reagent with a protease inhibitor cocktail. Homogenates were centrifuged at 15,000 rpm for 15 min, and supernatants were used for Western blot analysis to examine the ADP-ribosylated protein expression in the

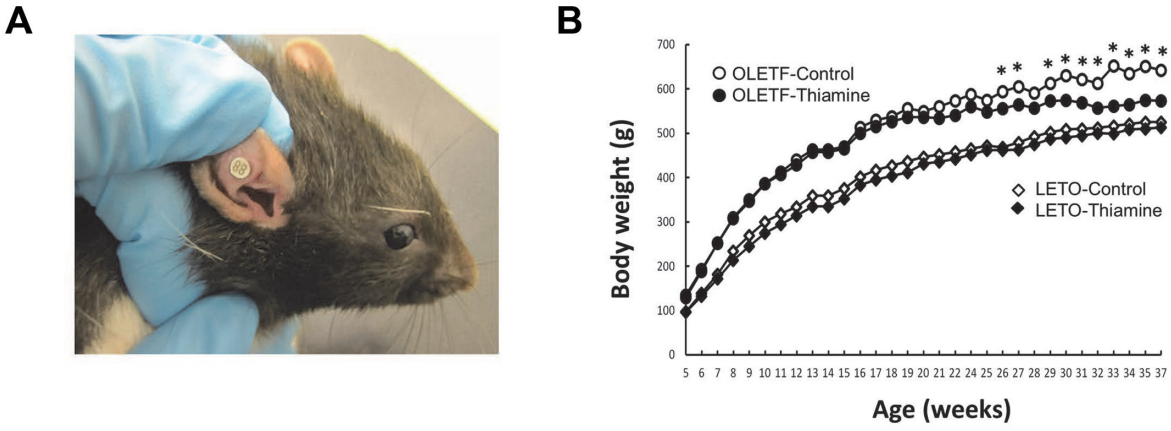

Fig. 1. Piercings indicating the individual numbers of the rats (A). Weekly body weights (B) from 5 to 37 weeks of age of Otsuka Long-Evans Tokushima Fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats unsupplemented or supplemented with 2 g of thiamine/L of drinking water for 33 weeks. The OLETF and LETO rats were randomly divided into the following groups: unsupplemented control OLETF group (OLETF-Control, n = 6), thiamine-supplemented OLETF group (OLETF-Thiamine, n = 6), unsupplemented control LETO group (LETO-Control, n = 3), and thiamine-supplemented LETO group (LETO-Thiamine, n = 3). Significant differences are noted between the OLETF-Control and OLETF-Thiamine groups at 26 to 37 weeks of age. *p < 0.05 compared with the OLETF-Control group.

liver.

Western blot analysis

Protein samples were separated by 4%-20% polyacrylamide gel electrophoresis, and proteins were transferred to polyvinylidene difluoride membranes. The membranes were blocked with a blocking buffer for 1 hr at room temperature. Then, the membranes were incubated with the specific primary anti-poly (ADP-ribose) polymer antibody in a signal enhancer solution (Nacalai Tesque, Kyoto, Japan) overnight at 4°C. Subsequently, the membranes were washed thrice with 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.1% Tween 20 to remove unbound antibodies. The membranes were then incubated with an HRP-conjugated secondary antibody in a signal enhancer solution (Nacalai Tesque) for 1 hr at room temperature. Chemiluminescence for ADP-ribosylated protein expression was detected with the Ez-Capture MG machine (ATTO Corp., Tokyo, Japan) using an enhanced chemiluminescence reagent (Nacalai Tesque).

Statistical analysis

Data are expressed as mean ± S.E. Group comparisons were performed using a two-tailed Student's *t*-test. Statistical analysis of the data from multiple groups was performed using ANOVA followed by Tukey tests. All statistical analyses were performed using Pharmaco Basic software (Scientist Press Co., Ltd., Tokyo, Japan). A p-value < 0.05 was considered statistically significant.

RESULTS

Body weight changes in the OLETF and LETO rats following thiamine supplementation

At the start of thiamine supplementation (5 weeks of age), the OLETF rats had similar body weights. Among the OLETF rats, a main obese effect on weekly body weight was markedly observed. The body weight of these rats was higher than that of the LETO rats. Notably, no differences in weekly body weights were observed between the thiamine-supplemented and unsupplemented LETO groups. On the other hand, substantial differences in weekly body weights were observed from the 20th week of thiamine supplementation until the end of 37 weeks of age between the thiamine-supplemented and unsupplemented OLETF groups. The body weights of rats in the thiamine-supplemented OLETF group were lower than the body weights of rats in the unsupplemented control OLETF group (Fig. 1B).

Effects of thiamine supplementation on casual and fasting blood glucose levels in the OLETF and LETO rats

The casual blood glucose levels tended to be higher in the unsupplemented control OLETF group than in the unsupplemented control LETO group throughout the experimental period; however, the differences were not significant (Fig. 2). Thiamine supplementation had no effect on the casual blood glucose levels in the OLETF and LETO rats (Fig. 2). The fasting blood glucose levels tended to be higher in the unsupplemented control OLETF group than in the unsupplemented control LETO group (Fig. 3). On the other hand, the fasting blood glucose levels were lower in the thiamine-supplemented OLETF group than in the unsupplemented control OLETF group (Fig. 3). Remarkably, the fasting blood glucose levels in the thiamine-supplemented OLETF group normalized to the levels in the unsupplemented control LETO group (Fig. 3).

Effect of thiamine supplementation on metabolic parameters in the OLETF and LETO rats

Thiamine supplementation substantially modulated metabolic parameters associated with obesity, diabe-

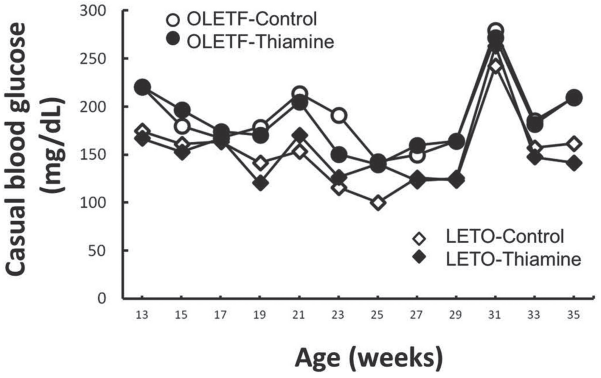

Fig. 2. Casual glucose levels in blood collected from the tail vein every 2 weeks from 13 to 35 weeks of age of Otsuka Long-Evans Tokushima Fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats unsupplemented or supplemented with 2 g of thiamine/L of drinking water for 33 weeks. The OLETF and LETO rats were randomly divided into the following groups: unsupplemented control OLETF group (OLETF-Control, n = 6), thiamine-supplemented OLETF group (OLETF-Thiamine, n = 6), unsupplemented control LETO group (LETO-Control, n = 2/3), and thiamine-supplemented LETO group (LETO-Thiamine, n = 2/3).

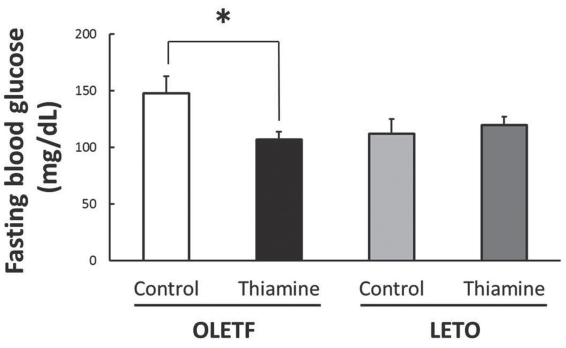

Fig. 3. Fasting glucose levels in blood collected from the tail vein following a 24-hr fast of 27-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats unsupplemented or supplemented with 2 g of thiamine/L of drinking water for 33 weeks. Each value represents mean ± S.E. n = 3-6 rats per group. *p < 0.05 compared with the unsupplemented control OLETF group.

tes, and hepatic disorders (Fig. 4). Plasma triglyceride and cholesterol levels were higher in the unsupplemented control OLETF group than in the unsupplemented control LETO group, and these levels were significantly lower in the thiamine-supplemented OLETF group than in the unsupplemented control OLETF group (Fig. 4A and 4B). Epididymal fat, retroperitoneal fat, and liver weights were higher in the unsupplemented control OLETF group than in the unsupplemented control LETO group (Fig. 4C, 4D and 4E), and these weights were significantly lower in the thiamine-supplemented OLETF group than in the unsupplemented control OLETF group (Fig. 4C, 4D and 4E).

Hepatic ADP-ribosylated protein expression in the OLETF rats

ADP-ribosylated protein expression was analyzed in the livers of the OLETF and LETO rats by Western blotting. An anti-poly (ADP-ribose) polymer antibody-positive band indicated the presence of ADP-ribosylated protein in the rat livers. Poly-ADP-ribosylated protein expression was higher in the unsupplemented control OLETF group than in the unsupplemented control LETO group (Fig. 5). Remarkably, hepatic poly-ADP-ribosylated protein expression was lower in the thiamine-supplemented OLETF group than in the unsupplemented control OLETF group (Fig. 5).

DISCUSSION

We previously reported that in obese diabetic rats having obesity-related diabetic complications, high-dose thiamine could suppress body weight gain and visceral fat mass increase and prevent fat accumulation in the liver (Tanaka *et al*., 2010; Kohda *et al*., 2012b). In this study, we found that thiamine supplementation profoundly decreased body weight, epididymal fat weight, and retroperitoneal fat weight in obese diabetic rats, suggesting effects associated with thiamine supplementation that modulated these metabolic parameters. In addition, thiamine supplementation substantially decreased the plasma triglyceride and cholesterol levels and liver weight in obese diabetic OLETF rats. As the liver significantly contributes to whole-body glucose homeostasis, it is a major target for the prevention and treatment of chronic hyperglycemia and resultant diabetes (Halter *et al*., 1985; Leclercq *et al*., 2007; Alkhalidy *et al*., 2018). Obesity-induced hepatic insulin resistance is a significant contributor to fasting hyperglycemia (Halter *et al*., 1985; Leclercq *et al*., 2007; Alkhalidy *et al*., 2018). In this study, thiamine had no effect on the casual blood glucose level in OLETF rats. Remarkably, the fasting blood glucose level in the thiamine-supplemented OLETF group normalized to the level in the unsupplemented control LETO group. Thiamine supplementation ameliorates fasting hyperglycemia probably through suppression of hepatic glucose production and enhancement of hepatic insulin sensitivity in obese diabetic rats. It is well recognized that obesity increases the risk of developing insulin resistance and type 2 diabetes mellitus (DeFronzo, 1997; Abdul-Ghani *et al*., 2008; Larsen, 2009). Additionally, NAFLD is a very common disorder among obese patients with diabetes (Adams, 2007). In this study, we found that thiamine supplementation prevented fasting hyperglycemia in OLETF rats, suggesting that high-dose thiamine may modify hepatic glucose metabolism and obesity-induced hepatic insulin resistance.

Substantial recent experimental studies have demonstrated the benefits of high-dose thiamine for diabetic complications (Babaei-Jadidi *et al*., 2003, 2004; Hammes *et al*., 2003; Thornalley, 2005; Beltramo *et al*., 2008). We previously reported that thiamine supplementation prevents obesity and diabetes-related liver disease (Tanaka *et al*., 2010; Kohda *et al*., 2012b). In fact, thiamine has a coenzymatic function in the glucose metabolic pathway; however, the biological and pharmacological relevance of high-dose thiamine supplementation remains unknown.

Adenosine thiamine triphosphate (AThTP) was recently identified in mouse brain, heart, skeletal muscle, kid-

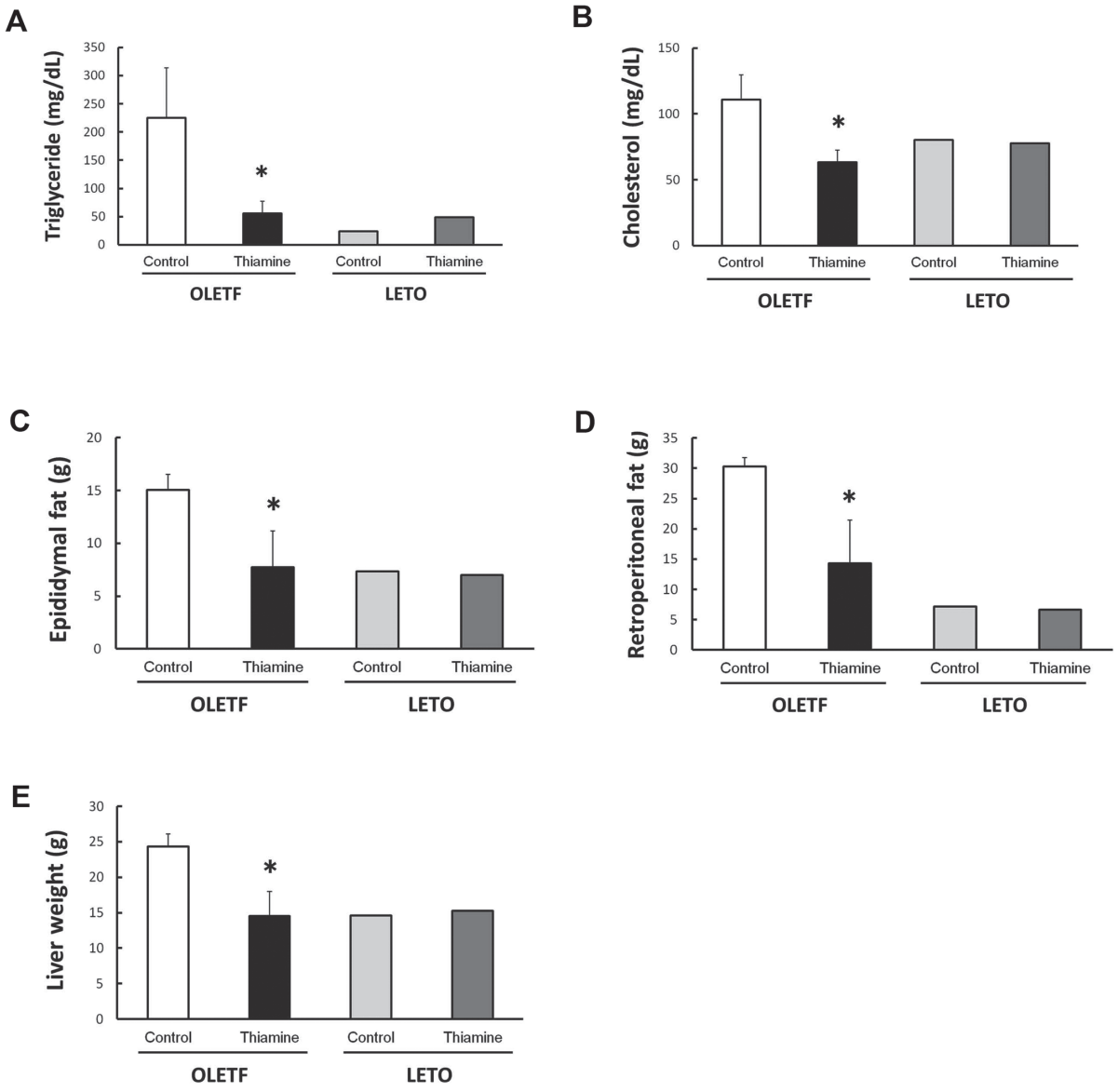

Fig. 4. Plasma triglyceride level (A), plasma cholesterol level (B), epididymal fat weight (C), retroperitoneal fat weight (D), and liver weight (E) of 38-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats unsupplemented or supplemented with 2 g of thiamine/L of drinking water for 33 weeks. Each value represents the mean ± S.E. n = 3 rats per OLETF group, n = 2 rats per LETO group.*$p < 0.05$ compared with the unsupplemented control OLETF group.

ney, and liver (Bettendorff *et al*., 2007; Frédérich *et al*., 2009). AThTP, a thiamine derivative, has previously been reported to significantly inhibit PARP-1 activation (Tanaka *et al*., 2011). In this study, we focused on protein modification, i.e., hepatic ADP-ribosylation that reflects PARP-1 activation and an increased oxidative stress condition. Obese diabetic OLETF rats exhibited increased ADP-ribosylated protein expression in the liver. Remarkably, hepatic ADP-ribosylated protein expression was attenuated with thiamine supplementation in OLETF rats. These results suggest that high-dose thiamine supplementation during the experimental period modulates oxidative stress by inhibiting hepatic ADP-ribosylation in obese diabetic OLETF rats. The beneficial effects of high-dose

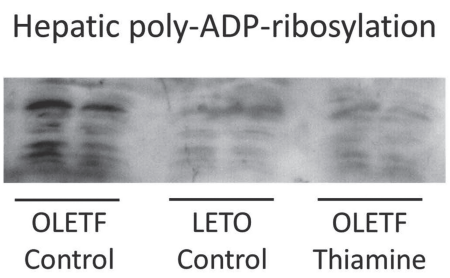

Fig. 5. Expression of adenosine diphosphate (ADP)-ribosylated protein in the livers of 38-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats unsupplemented or supplemented with 2 g of thiamine/L of drinking water for 33 weeks. Liver samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by Western blot analysis with a specific antibody against the poly (ADP-ribose) polymer. The antibody-positive bands indicate the presence of poly-ADP-ribosylated protein in the liver.

thiamine on oxidative stress, obesity, and diabetes-related liver diseases may be attributable to its inhibitory effect on PARP-1 activation in addition to its role as a coenzyme. Further investigation is warranted on the existence of noncoenzymatic functions of high-dose thiamine.

In the future, we aim to answer the novel research question "Can high-dose thiamine supplementation lead to the production of AThTP, a thiamine derivative?" We plan to elucidate the effects of high-dose thiamine on obesity and diabetic complications by producing the thiamine derivative AThTP with inhibitory action on PARP-1 activation. In addition, we plan to examine the hepatoprotective and obesity-preventive effects of the thiamine derivative AThTP expressed in the liver to establish a method to prevent and treat oxidative stress-related diseases.

In conclusion, our results suggest that thiamine supplementation modulates oxidative stress by inhibiting hepatic ADP-ribosylation.

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

REFERENCES

Abdul-Ghani, M.A., Matsuda, M., Jani, R., Jenkinson, C.P., Coletta, D.K., Kaku, K. and DeFronzo, R.A. (2008): The relationship between fasting hyperglycemia and insulin secretion in subjects with normal or impaired glucose tolerance. Am. J. Physiol. Endocrinol. Metab., **295**, E401-E406.

Adams, L.A. and Angulo, P. (2005): Recent concepts in non-alcoholic fatty liver disease. Diabet. Med., **22**, 1129-1133.

Adams, L.A. (2007): Nonalcoholic fatty liver disease and diabetes mellitus. Endocr. Res., **32**, 59-69.

Alkhalidy, H., Moore, W., Wang, A., Luo, J., McMillan, R.P., Wang, Y., Zhen, W., Hulver, M.W. and Liu, D. (2018): Kaempferol ameliorates hyperglycemia through suppressing hepatic gluconeogenesis and enhancing hepatic insulin sensitivity in diet-induced obese mice. J. Nutr. Biochem., **58**, 90-101.

Argo, C.K. and Caldwell, S.H. (2009): Epidemiology and natural history of non-alcoholic steatohepatitis. Clin. Liver Dis., **13**, 511-531.

Arrese, M. (2010): Nonalcoholic fatty liver disease: liver disease: an overlooked complication of diabetes mellitus. Nat. Rev. Endocrinol., **6**, 660-661.

Babaei-Jadidi, R., Karachalias, N., Ahmed, N., Battah, S. and Thornalley, P.J. (2003): Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. Diabetes, **52**, 2110-2120.

Babaei-Jadidi, R., Karachalias, N., Kupich, C., Ahmed, N. and Thornalley, P.J. (2004): High-dose thiamine therapy counters dyslipidaemia in streptozotocin-induced diabetic rats. Diabetologia, **47**, 2235-2246.

Beltramo, E., Berrone, E., Tarallo, S. and Porta, M. (2008): Effects of thiamine and benfotiamine on intracellular glucose metabolism and relevance in the prevention of diabetic complications. Acta Diabetol., **45**, 131-141.

Bellentani, S. and Marino, M. (2009): Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). Ann. Hepatol., **8**, S4-S8.

Bellentani, S., Scaglioni, F., Marino, M. and Bedogni, G. (2010): Epidemiology of non-alcoholic fatty liver disease. Dig. Dis., **28**, 155-161.

Bettendorff, L., Wirtzfeld, B., Makarchikov, A.F., Mazzucchelli, G., Frédérich, M., Gigliobianco, T., Gangolf, M., De Pauw, E., Angenot, L. and Wins, P. (2007): Discovery of a natural thiamine adenine nucleotide. Nat. Chem. Biol., **3**, 211-212.

Beyer, A.M. and Weihrauch, D. (2012): Hexosamine pathway activation and O-linked-N-acetylglucosamine: novel mediators of endothelial dysfunction in hyperglycemia and diabetes. Vascul. Pharmacol., **56**, 113-114.

Chapple, I.L. and Matthews, J.B. (2007): The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol. 2000, **43**, 160-232.

Chung, S.S., Ho, E.C., Lam, K.S. and Chung, S.K. (2003): Contribution of polyol pathway to diabetes-induced oxidative stress. J. Am. Soc. Nephrol., **14**, S233-S236.

Cusi, K. (2009): Nonalcoholic fatty liver disease in type 2 diabetes mellitus. Curr. Opin. Endocrinol. Diabetes Obes., **16**, 141-149.

Daniels, C.M., Ong, S.E. and Leung, A.K. (2015): The promise of proteomics for the study of ADP-ribosylation. Mol. Cell, **58**, 911-924.

DeFronzo, R.A. (1997): Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. Neth. J. Med., **50**, 191-197.

Del Prato, S. (2009): Role of glucotoxicity and lipotoxicity in the pathophysiology of Type 2 diabetes mellitus and emerging treatment strategies. Diabet. Med., **26**, 1185-1192.

Dunlop, M. (2000): Aldose reductase and the role of the polyol pathway in diabetic nephropathy. Kidney Int. Suppl., **77**, S3-S12.

Evans, J.L., Goldfine, I.D., Maddux, B.A. and Grodsky, G.M. (2003): Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes, **52**, 1-8.

Frédérich, M., Delvaux, D., Gigliobianco, T., Gangolf, M., Dive, G., Mazzucchelli, G., Elias, B., De Pauw, E., Angenot, L., Wins, P. and Bettendorff, L. (2009): Thiaminylated adenine nucleotides. Chemical synthesis, structural characterization and natural occurrence. FEBS J., **276**, 3256-3268.

Halter, J.B., Ward, W.K., Porte, D. Jr., Best, J.D. and Pfeifer, M.A. (1985): Glucose regulation in non-insulin-dependent diabetes mellitus. Interaction between pancreatic islets and the liver. Am. J. Med., **79**, 6-12.

Hammes, H.P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Ju, Q., Lin, J., Bierhaus, A., Nawroth, P., Hannak, D., Neumaier, M., Bergfeld, R., Giardino, I. and Brownlee, M. (2003): Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat. Med., **9**, 294-299.

Kaiser, N., Leibowitz, G. and Nesher, R. (2003): Glucotoxicity and beta-cell failure in type 2 diabetes mellitus. J. Pediatr. Endocrinol. Metab., **16**, 5-22.

Kohda, Y., Shirakawa, H., Yamane, K., Otsuka, K., Kono, T., Terasaki, F. and Tanaka, T. (2008): Prevention of incipient diabetic cardiomyopathy by high-dose thiamine. J. Toxicol. Sci., **33**, 459-472.

Kohda, Y., Kanematsu, M., Kono, T., Terasaki, F. and Tanaka, T. (2009): Protein *O*-glycosylation induces collagen expression and contributes to diabetic cardiomyopathy in rat cardiac fibroblasts. J. Pharmacol. Sci., **111**, 446-450.

Kohda, Y., Tanaka, T. and Matsumura, H. (2012a): Effect of thiamine repletion on cardiac fibrosis and protein *O*-glycosylation in diabetic cardiomyopathy. J. Diabetes Metab. **S7**, 001

Kohda, Y., Tanaka, T. and Matsumura, H. (2012b): Role of thiamine in obesity-related diabetes: Modification of the gene expression. Food and Nutritional Components in Focus No.4, B Vitamins and Folate: Chemistry, Analysis, Function and Effects. The Royal Society of Chemistry, 580-591.

Kohda, Y., Maekita, A., Tanaka, T. and Matsumura, H. (2017): Hepatic glucose-dependent insulinotropic polypeptide expression is modified by supplementing high-dose thiamine in obese diabetic rats. Fundam. Toxicol. Sci., **4**, 279-284.

Larsen, M.O. (2009): Beta-cell function and mass in type 2 diabetes. Dan. Med. Bull., **56**, 153-164.

Lazo, M. and Clark, J.M. (2008): The epidemiology of nonalcoholic fatty liver disease: a global perspective. Semin. Liver Dis., **28**, 339-350.

Leclercq, I.A., Da Silva Morais, A., Schroyen, B., Van Hul, N. and Geerts, A. (2007): Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. J. Hepatol., **47**, 142-156.

Luo, X. and Kraus, W.L. (2012): On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev., **26**, 417-432.

Masutani, M., Suzuki, H., Kamada, N., Watanabe, M., Ueda, O., Nozaki, T., Jishage, K., Watanabe, T., Sugimoto, T., Nakagama, H., Ochiya, T. and Sugimura, T. (1999): Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA, **96**, 2301-2304.

Moscatiello, S., Manini, R. and Marchesini, G. (2007): Diabetes and liver disease: an ominous association. Nutr. Metab. Cardiovasc. Dis., **17**, 63-70.

Pacher, P. and Szabó, C. (2005): Role of poly(ADP-ribose) polymerase-1 activation in the pathogenesis of diabetic complications: endothelial dysfunction, as a common underlying theme. Antioxid. Redox Signal., **7**, 1568-1580.

Pieper, A.A., Brat, D.J., Krug, D.K., Watkins, C.C., Gupta, A., Blackshaw, S., Verma, A., Wang, Z.Q. and Snyder, S.H. (1999): Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA, **96**, 3059-3064.

Poitout, V. and Robertson, R.P. (2002): Minireview: secondary beta-cell failure in type 2 diabetes--a convergence of glucotoxicity and lipotoxicity. Endocrinology, **143**, 339-342.

Porepa, L., Ray, J.G., Sanchez-Romeu, P. and Booth, G.L. (2010): Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. CMAJ, **182**, E526-E531.

Roseman, H.M. (2005): Progression from obesity to type 2 diabetes: lipotoxicity, glucotoxicity, and implications for management. J. Manag. Care Pharm., **11**, S3-S11.

Szabó, C. (2005): Roles of poly(ADP-ribose) polymerase activation in the pathogenesis of diabetes mellitus and its complications. Pharmacol. Res., **52**, 60-71.

Tanaka, T., Kono, T., Terasaki, F., Yasui, K., Soyama, A., Otsuka, K., Fujita, S., Yamane, K., Manabe, M., Usui, K. and Kohda, Y. (2010): Thiamine prevents obesity and obesity-associated metabolic disorders in OLETF rats. J. Nutr. Sci. Vitaminol. (Tokyo), **56**, 335-346.

Tanaka, T., Yamamoto, D., Sato, T., Tanaka, S., Usui, K., Manabe, M., Aoki, Y., Iwashima, Y., Saito, Y., Mino, Y. and Deguchi, H. (2011): Adenosine thiamine triphosphate (AThTP) inhibits poly(ADP-ribose) polymerase-1 (PARP-1) activity. J. Nutr. Sci. Vitaminol. (Tokyo), **57**, 192-196.

Thornalley, P.J. (2005): The potential role of thiamine (vitamin B1) in diabetic complications. Curr. Diabetes Rev., **1**, 287-298.

Virág, L. and Szabó, C. (2002): The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol. Rev., **54**, 375-429.

Vlassara, H. and Striker, G.E. (2013): Advanced glycation endproducts in diabetes and diabetic complications. Endocrinol. Metab. Clin. North Am., **42**, 697-719.

Waddington, R.J., Moseley, R. and Embery, G. (2000): Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral Dis., **6**, 138-151.

Weir, G.C., Marselli, L., Marchetti, P., Katsuta, H., Jung, M.H. and Bonner-Weir, S. (2009): Towards better understanding of the contributions of overwork and glucotoxicity to the beta-cell inadequacy of type 2 diabetes. Diabetes Obes. Metab., **11**, 82-90.

Wolff, S.P., Jiang, Z.Y. and Hunt, J.V. (1991): Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic. Biol. Med., **10**, 339-352.

Yan, L.J. (2014): Pathogenesis of chronic hyperglycemia: from reductive stress to oxidative stress. J. Diabetes Res., 137919.