Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.4, No.6, 279-284, 2017

The Japanese Society of Toxicology

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

Original Article

Hepatic glucose-dependent insulinotropic polypeptide expression is modified by supplementing high-dose thiamine in obese diabetic rats

Yuka Kohda, Akie Maekita, Takao Tanaka and Hitoshi Matsumura

Laboratory of Pharmacotherapy, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan

(Received December 14, 2017; Accepted December 15, 2017)

ABSTRACT — Glucose toxicity and lipotoxicity are important states in obesity and diabetes. We previously reported that thiamine supplementation decreases body weight and visceral fat mass in rats with obesity-related diabetes. Glucose-dependent insulinotropic polypeptide (GIP) acts on pancreatic β cells to promote insulin secretion. According to established theory, GIP is derived from the gastrointestinal tract. We previously discovered increased expression of the GIP gene in the livers of obese rats with diabetes receiving high-dose thiamine. We referred to our previous dataset of gene expression analysis using a microarray for livers, which led to the new idea for the present study. We focused on "liver-derived GIP" to demonstrate GIP protein expression in the liver and visually present localization of GIP in the liver. Four-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats were randomly divided into two groups: an unsupplemented control group and a thiamine-supplemented group receiving 2 g of thiamine/L in drinking water for 51 weeks. GIP protein expression in the livers of OLETF rats at 55 weeks of age were determined by western blotting and immunohistochemical analysis. GIP protein expression in the liver was increased in thiamine-supplemented rats compared with that in controls, suggesting that it is involved in preventing and controlling obesity-related diabetic complications. The novel findings of this study that GIP is expressed in the liver, is likely to be added to the story regarding GIP modification of the obese diabetic state.

Key words: Thiamine supplementation, Hepatic GIP expression, Glycolipid toxicity, Obesity, Diabetic complications

INTRODUCTION

Glucose toxicity and lipotoxicity are important states in obesity and diabetes. Insulin resistance is influenced by diet, body weight, increased levels of triglycerides and obesity. The prevalence of obesity continues to markedly increase worldwide (Ng *et al*., 2014; The GBD 2015 Obesity Collaborators, 2017). The global epidemic of obesity also heralds an epidemic of type 2 diabetes, which has escalated health care costs related to the burden of associated complications (NCD Risk Factor Collaboration, 2016). Obesity is linked with type 2 diabetes in terms of increasing the risk of developing type 2 diabetes and that of its associated morbidity (NCD Risk Factor Collaboration, 2016). We previously reported that thiamine (vitamin B1) treatment decreased body weight, visceral fat mass and adipocyte size in obesity-related diabetic rats (Tanaka *et al*., 2010; Kohda *et al*., 2012b). By definition, vitamins are exogenous organic compounds required in small amounts for metabolic processes. Thi-

Correspondence: Yuka Kohda (E-mail: ykohda@gly.oups.ac.jp)

amine functions as a cofactor in the decarboxylation of keto acids and interconversion of glucose. It is widely found in foods but is abundant in relatively few, including pork, as well as grains and seeds with intact bran (Turck *et al*., 2016). Of note, heat during food preparation may affect thiamine function.

Several studies have reported potential therapeutic applications for thiamine in treating diabetic complications (Raval *et al*., 2015; Kohda *et al*., 2008, 2009, 2010, 2012a; Tanaka *et al*., 2010). *In vitro* studies have demonstrated that thiamine decreases apoptosis induced by high glucose conditioned extracellular matrix in human retinal pericytes, and a pilot study supported the use of high-dose thiamine as a potential treatment for end-stage diabetic nephropathy (Rabbani *et al*., 2009; Beltramo *et al*., 2009).

Using obese diabetic rats, we previously reported that diabetic cardiomyopathy was alleviated by supplementing high-dose thiamine (Tanaka *et al*., 2010). Our previous gene expression analysis of the livers of obese diabetic rats that received high-dose thiamine by microarray demonstrated the increased expression of the incretin glucose-dependent insulinotropic polypeptide (GIP) gene (Tanaka *et al*., 2010; Kohda *et al*., 2012b). Body weight gain and increased visceral fat mass in obese diabetic rats were suppressed by high-dose thiamine supplementation (Tanaka *et al*., 2010). Although GIP is thought to be an obesity hormone or a cause of type 2 diabetes (Marks, 2006), we discovered that GIP gene expression was increased in the liver, despite decreased fat accumulation and obesity (Kohda *et al*., 2012b).

Incretin GIP, secreted from the small intestine, acts on pancreatic β cells to promote insulin secretion (Gautier *et al*., 2005; Baggio and Drucker, 2007). According to the established theory, GIP derives from the gastrointestinal tract. Interestingly, we found that GIP gene expression was increased in the rat liver (Fig. 1, Modified from Kohda *et al*., 2012b). We referred to our previous dataset of gene expression analysis using a microarray for livers (Tanaka *et al*., 2010; Kohda *et al*., 2012b), which led to the new idea for the present study.

We are setting a new research question "Does liver-derived incretin GIP exist?" In the present study, we advance the findings of our previous study by focusing on the location of GIP protein expression in the liver.

MATERIALS AND METHODS

Chemicals

Thiamine hydrochloride was supplied by Kishida Chemical Co., Ltd (Osaka, Japan). Antibodies used were as follows: anti-GIP, anti-β-actin, horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Mammalian tissue lysis and extraction reagent was purchased from Sigma (MO, USA). Protease inhibitor cocktail and phosphatase inhibitor cocktail were supplied by Nacalai Tesque (Kyoto, Japan). All other chemicals used were of

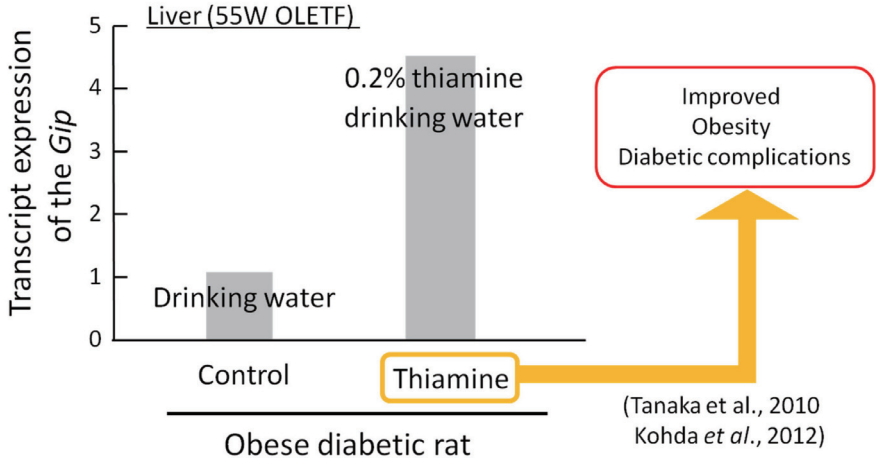

Fig. 1. Focusing on the glucose-dependent insulinotropic polypeptide (GIP) expression in the liver. Increased expression of the GIP gene in the livers of Otsuka Long-Evans Tokushima Fatty (OLETF) rats receiving 0.2% thiamine in the drinking water for 51 weeks. Transcript expression of the *Gip* was analyzed by microarray. Transcript expression of the *Gip* in livers of high-dose thiamine-supplemented rats was higher than in control OLETF rats at 55 weeks (55W) of age. Modified from Tanaka *et al*., 2010; Kohda *et al*., 2012b.

the highest purity and were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and experimental design

We previously found that thiamine mitigates obesity and diabetic complications in Otsuka Long-Evans Tokushima Fatty (OLETF) rats that exhibit progressive obesity and metabolic disorders (Tanaka *et al*., 2010). In this experiment, we used a liver tissue sampling frame previously described (Tanaka *et al*., 2010).

Four-week-old male OLETF rats were randomly divided into two groups, an unsupplemented control group and a thiamine-supplemented group. The high-dose thiamine-supplemented group received 2 g thiamine/L in drinking water for 51 weeks. Experimental protocols and animal care methods were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

Preparation of the protein extraction solution from rat livers

A liver weight of 300 mg tissue was homogenized at 4°C in 450 μL tissue lysis and extraction reagent with a protease inhibitor cocktail and a phosphatase inhibitor cocktail. Homogenates were centrifuged at 15,000 rpm for 15 min, and supernatants were used for western blot analysis to examine the expression of GIP protein. Protein determinations were performed using Lowry's method (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a standard.

Western blot analysis

Protein samples were separated by 10-20% gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to polyvinylidene difluoride membranes. Membranes were blocked with 0.3% skimmed milk in buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.1% Tween 20 (TBST) for 1 h at room temperature. The membranes were then incubated with the specific primary antibodies anti-GIP and anti-β-actin in TBST overnight at 4°C. Membranes were washed 3 times in TBST to remove unbound antibodies. They were then incubated with HRP-conjugated secondary antibody in TBST for 1 hr at room temperature. Chemiluminescence was detected by a LAS-3000 machine (Fuji Film Corp., Tokyo) using an enhanced chemiluminescence reagent (Chemi-Lumi One; Nacalai Tesque, Kyoto). The expression of β-actin was used as an internal standard.

Immunohistochemistry

In this experiment, we used the liver tissue sampling frame as previously reported for OLETF rats. Briefly, the liver organs were removed, immediately fixed in 10% phosphate-buffered formalin solution and then embedded in paraffin. Paraffin-embedded sections were immunohistochemically stained to study the localization of GIP in the liver at a contracted pathological laboratory (Kyodo Byori Inc., Kobe, Japan). Anti-GIP antibody was used to determine whether GIP protein was present in the small intestine.

Statistical analysis

Statistical analyses were performed using the Microsoft Excel Data Analysis ToolPac (Statcel2, OMS Publish Inc., Tokyo, Japan). Group comparisons were performed using a 2-tailed Student's *t*-test. $P < 0.05$ was considered statistically significant. Data are expressed as the mean ± S.E.

RESULTS

Hepatic GIP protein expression in obese diabetic rats during the suppression of obesity

Although we previously reported that the GIP gene is expressed in the livers of OLETF rats (Kohda *et al*., 2012b), no report has been published on GIP protein expression in the liver of obese diabetic rats. Therefore, in the present study, GIP protein expression was analyzed in the livers of obese diabetic rats by western blotting. An anti-GIP antibody-positive band indicating GIP protein was detected in control rat livers and in rats that received high-dose thiamine supplementation (Fig. 2A). GIP protein expression was increased in thiamine-supplemented rats despite the suppression of obesity and diabetic complications (Fig. 2B).

Effect of thiamine on the localization of GIP protein in the liver of obese diabetic rats

GIP was immunohistochemically stained to visualize its localization in the liver. The present study clearly demonstrated the localization of GIP in the livers of obese diabetic OLETF rats. At the age of 55 weeks, high-dose thiamine-supplemented OLETF rats had increased GIP protein expression compared with control OLETF rats (Fig. 3A and 3B).

DISCUSSION

In the present study, we focused on obesity and GIP expression in the livers of diabetic rats. In obese diabet-

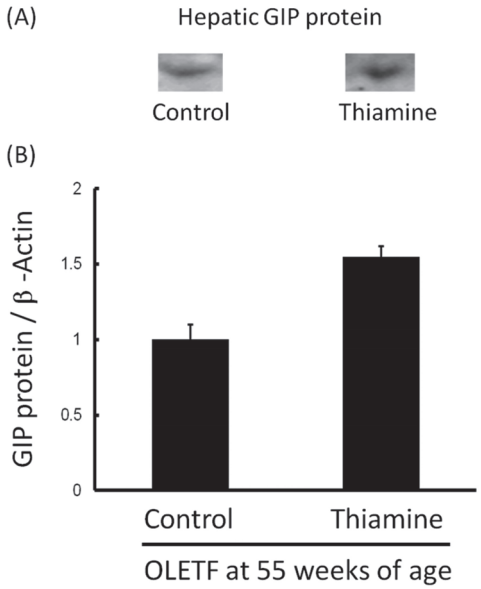

Fig. 2. Glucose-dependent insulinotropic polypeptide (GIP) expression of the liver in control and thiamine-supplemented Otsuka Long-Evans Tokushima Fatty (OLETF) rats at 55 weeks of age. Liver samples were subjected to SDS-PAGE followed by western blot analysis with a specific antibody against GIP (A). Data were normalized by β-actin expression and expressed as arbitrary units (B). Thiamine-supplemented groups received 0.2% thiamine in the drinking water for 51 weeks. Each value represents the mean ± S.E. n = 4 per group.

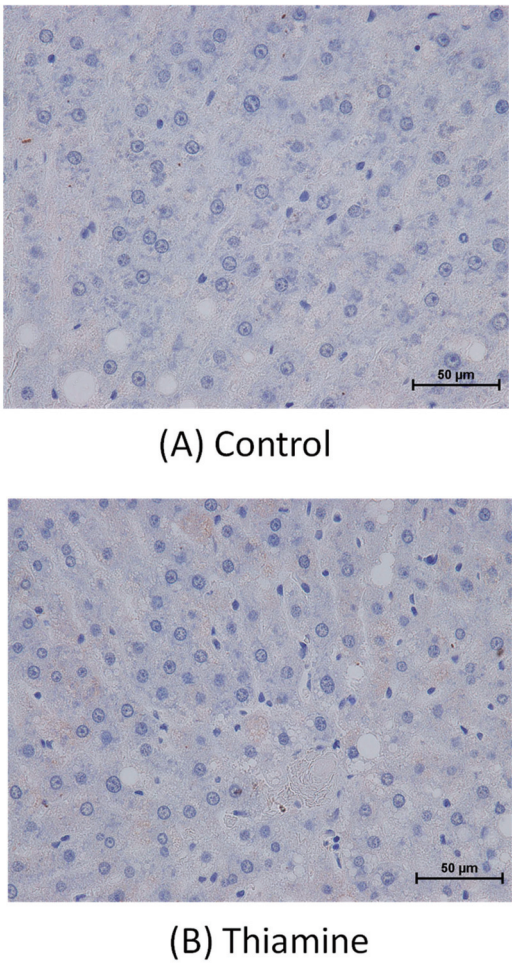

Fig. 3. Effect of thiamine supplementation on glucose-dependent insulinotropic polypeptide (GIP) protein expression in the livers of obese diabetic rats. Immunohistochemistry assay was performed to detect the protein expression of GIP in the livers of OLETF rats. At 55 weeks of age, high-dose thiamine-supplemented OLETF rats (B) had increased GIP protein expression compared with water treated OLETF rats (A). The thiamine-supplemented group received 0.2% thiamine in the drinking water for 51 weeks. Scale bar = 50 μm.

ic rats suffering from obesity-related diabetic complications, body weight gain and increased visceral fat mass were suppressed and fat accumulation in the livers was alleviated by thiamine supplementation (Tanaka *et al.*, 2010). Our previous study demonstrated the increased expression of the GIP gene in the livers of thiamine-supplemented rats (Kohda *et al.*, 2012b). In addition, we previously reported that the streptozotocin-induced diabetic state triggered GIP expression in rat livers (Kohda *et al.*, 2016). GIP and GIP receptor expressions in the retinas of diabetic rats, as previously reported, suggest GIP is derived from tissues other than the gastrointestinal tract (Cho *et al.*, 2002). During the gradual impairment of mouse pancreatic β cells, GIP alleviates the impaired insulin secretion, suggesting the existence of pancreatic GIP as an incretin not derived from the gastrointestinal tract (Iida *et al.*, 2016). However, no report has reported GIP expression in the liver of obese diabetic rats. Therefore, in the present study, GIP protein expression was analyzed in the livers of obese diabetic rats by western blotting. An anti-GIP antibody-positive band was detected in control and thiamine-supplemented, suggesting the existence of hepatic GIP. Hepatic GIP protein expression was increased in thiamine-supplemented rats compared with controls. Immunohistochemical analysis indicated GIP was expressed in the liver. Thus, GIP gene and protein expression in the liver may modify and be involved in the improvement of obesity-related diabetic complications.

GIP is also thought to be an obesity hormone and cause of type 2 diabetes, because increased GIP signaling promotes fat accumulation in adipose tissue through

lipid oxidation (Mark, 2006). Our previous study demonstrated that GIP gene expression was increased in the livers of obese diabetic rats, despite the alleviation of obesity, by supplementing with high-dose thiamine (Tanaka *et al*., 2010; Kohda *et al*., 2012b). To date, no study has reported the effects of GIP on liver function. Incretin GIP derived from the gastrointestinal tract has fat-accumulating effects on adipocytes, and, therefore, is called an obesity hormone (Yip and Wolfe, 2000; Getty-Kaushik *et al*., 2006). As previously reported, GIP activates lipoprotein lipase (Kim *et al*., 2007), suggesting that GIP expressed in the liver may be involved in gluco-lipid metabolism.

The current study reports a new concept related to the expression of GIP in the liver of obese diabetic rats. In future studies, we will elucidate the mechanism of GIP expression in the liver and the effects of GIP on obesity and diabetic complications. In addition, we will examine the hepatoprotective and obesity-preventing effects of GIP expressed in the liver to establish a method to prevent and treat obesity, diabetes-related liver diseases, and diabetic complications.

Gut-derived incretin GIP enhances insulin secretion. The relationship between GIP expression, likely to be derived from the liver, and insulin secretion remains unknown, necessitating further study. Interestingly, we reported that GIP was expressed only in the livers of streptozotocin-treated rats under non-hyperglycemic conditions (Kohda *et al*., 2016). We would like to examine the effects of liver GIP expression on pancreatic β cells and livers in the obese-diabetic state. Liver-derived incretin GIP should be further investigated to clarify its mechanisms to improve pancreatic β-cell functions, normalize insulin secretion, and achieve blood glucose control in obese diabetes, thereby establishing a method to prevent and treat obesity and diabetic complications by targeting the liver. The absorption of thiamine from food, dietary supplements and medicines might be important in preventing and controlling obesity-related metabolic complications.

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

REFERENCES

Baggio, L.L. and Drucker, D.J. (2007): Biology of incretins: GLP-1 and GIP. Gastroenterology, **132**, 2131-2157.

Beltramo, E., Nizheradze, K., Berrone, E., Tarallo, S., Porta, M. (2009): Thiamine and benfotiamine prevent apoptosis induced by high glucose-conditioned extracellular matrix in human retinal pericytes. Diabetes Metab. Res. Rev., **25**, 647-656.

Cho, G.J., Ryu, S., Kim, Y.H., Kim, Y.S., Cheon, E.W., Park, J.M., Kim, H.J., Kang, S.S. and Choi, W.S. (2002): Upregulation of glucose-dependent insulinotropic polypeptide and its receptor in the retina of streptozotocin-induced diabetic rats. Curr. Eye Res., **25**, 381-388.

Gautier, J.F., Fetita, S., Sobngwi, E. and Salaün-Martin, C. (2005): Biological actions of the incretins GIP and GLP-1 and therapeutic perspectives in patients with type 2 diabetes. Diabetes Metab., **31**, 233-242.

Getty-Kaushik, L., Song, D.H., Boylan, M.O., Corkey, B.E. and Wolfe, M.M. (2006): Glucose-dependent insulinotropic polypeptide modulates adipocyte lipolysis and reesterification. Obesity. **14**, 1124-1131.

Iida, A., Seino, Y., Fukami, A., Maekawa, R., Yabe, D., Shimizu, S., Kinoshita, K., Takagi, Y., Izumoto, T., Ogata, H., Ishikawa, K., Ozaki, N., Tsunekawa, S., Hamada, Y., Oiso, Y., Arima, H. and Hayashi, Y. (2016): Endogenous GIP ameliorates impairment of insulin secretion in proglucagon-deficient mice under moderate beta cell damage induced by streptozotocin. Diabetologia, **59**, 1533-1541.

Kim, S.J., Nian, C. and McIntosh, C.H. (2007): Activation of lipoprotein lipase by glucose-dependent insulinotropic polypeptide in adipocytes. A role for a protein kinase B, LKB1, and AMP-activated protein kinase cascade. J. Biol. Chem., **282**, 8557-8567.

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., Umeki, M., Kono, T., Terasaki, F., Matsumura, H. and Tanaka, T. (2010): Thiamine ameliorates diabetes-induced inhibition of pyruvate dehydrogenase (PDH) in rat heart mitochondria: investigating the discrepancy between PDH activity and PDH E1α phosphorylation in cardiac fibroblasts exposed to high glucose. J. Pharmacol. Sci., **113**, 343-352.

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., Minamigawa, C., Matsuo, M. and Matsumura, H. (2016): Streptozotocin-induced diabetic state triggers glucose-dependent insulinotropic polypeptide (GIP) expression in the rat liver. Fundam. Toxicol. Sci., **3**, 291-296.

Marks, V. (2006): Human obesity: its hormonal basis and the role of gastric inhibitory polypeptide. Med. Princ. Pract., **15**, 325-337.

NCD Risk Factor Collaboration (NCD-RisC). (2016): Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet., **387**,1513-1530.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F., Abraham, J.P., Abu-Rmeileh, N.M., Achoki, T., AlBuhairan, F.S., Alemu, Z.A., Alfonso, R., Ali, M.K., Ali, R., Guzman, N.A., Ammar, W., Anwari, P., Banerjee, A., Barquera, S., Basu, S., Bennett, D.A., Bhutta, Z., Blore, J., Cabral, N., Nonato, I.C., Chang, J.C., Chowdhury, R., Courville, K.J.,

Criqui, M.H., Cundiff, D.K., Dabhadkar, K.C., Dandona, L., Davis, A., Dayama, A., Dharmaratne, S.D., Ding, E.L., Durrani, A.M., Esteghamati, A., Farzadfar, F., Fay, D.F., Feigin, V.L., Flaxman, A., Forouzanfar, M.H., Goto, A., Green, M.A., Gupta, R., Hafezi-Nejad, N., Hankey, G.J., Harewood, H.C., Havmoeller, R., Hay, S., Hernandez, L., Husseini, A., Idrisov, B.T., Ikeda, N., Islami. F., Jahangir, E., Jassal, S.K., Jee, S.H., Jeffreys, M., Jonas, J.B., Kabagambe, E.K., Khalifa, S.E., Kengne, A.P., Khader, Y.S., Khang, Y.H., Kim, D., Kimokoti, R.W., Kinge, J.M., Kokubo, Y., Kosen, S., Kwan, G., Lai, T., Leinsalu, M., Li, Y., Liang, X., Liu, S., Logroscino, G., Lotufo, P.A., Lu, Y., Ma, J., Mainoo, N.K., Mensah, G.A., Merriman, T.R., Mokdad, A.H., Moschandreas, J., Naghavi, M., Naheed, A., Nand, D., Narayan, K.M., Nelson, E.L., Neuhouser, M.L., Nisar, M.I., Ohkubo, T., Oti, S.O., Pedroza, A., Prabhakaran, D., Roy, N., Sampson, U., Seo, H., Sepanlou, S.G., Shibuya, K., Shiri, R., Shiue, I., Singh, G.M., Singh, J.A., Skirbekk, V., Stapelberg, N.J., Sturua, L., Sykes, B.L., Tobias, M., Tran, B.X., Trasande, L., Toyoshima, H., van de Vijver, S., Vasankari, T.J., Veerman, J.L., Velasquez-Melendez, G., Vlassov, V.V., Vollset, S.E., Vos, T., Wang, C., Wang, X., Weiderpass, E., Werdecker, A., Wright, J.L., Yang, Y.C., Yatsuya, H., Yoon, J., Yoon, S.J., Zhao, Y., Zhou, M., Zhu, S., Lopez, A.D., Murray, C.J. and Gakidou E. (2014): Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study. Lancet., **384**, 766-781.

Rabbani, N., Alam, S.S., Riaz, S., Larkin, J.R., Akhtar, M.W., Shafi, T. and Thornalley, P.J. (2009): High-dose thiamine therapy for patients with type 2 diabetes and microalbuminuria: a randomised, double-blind placebo-controlled pilot study. Diabetologia. **52**, 208-212.

Raval, A.D., Thakker, D., Rangoonwala, A.N., Gor. D. and Walia, R. (2015): Vitamin B and its derivatives for diabetic kidney disease. Cochrane. Database. Syst. Rev., **1**, CD009403. doi:10.1002/14651858.CD009403.pub2.

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., **56**, 335-346.

The GBD 2015 Obesity Collaborators, Afshin, A., Forouzanfar, M.H., Reitsma, M.B., Sur, P., Estep, K., Lee, A., Marczak, L., Mokdad, A.H., Moradi-Lakeh, M., Naghavi, M., Salama, J.S., Vos, T., Abate, K.H., Abbafati, C., Ahmed, M.B., Al-Aly, Z., Alkerwi, A., Al-Raddadi, R., Amare, A.T., Amberbir, A., Amegah, A.K., Amini, E., Amrock, S.M., Anjana, R.M., Ärnlöv, J., Asayesh, H., Banerjee, A., Barac, A., Baye, E., Bennett, D.A., Beyene, A.S., Biadgilign, S., Biryukov, S., Bjertness, E., Boneya, D.J., Campos-Nonato, I., Carrero, J.J., Cecilio, P., Cercy, K., Ciobanu, L.G., Cornaby, L., Damtew, S.A., Dandona, L., Dandona, R., Dharmaratne, S.D., Duncan, B.B., Eshrati, B., Esteghamati, A., Feigin, V.L., Fernandes, J.C., Fürst, T., Gebrehiwot, T.T., Gold, A., Gona, P.N., Goto, A., Habtewold, T.D., Hadush, K.T., Hafezi-Nejad, N., Hay, S.I., Horino, M., Islami, F., Kamal, R., Kasaeian, A., Katikireddi, S.V., Kengne, A.P., Kesavachandran, C.N., Khader, Y.S., Khang, Y.H., Khubchandani, J., Kim, D., Kim, Y.J., Kinfu, Y., Kosen S., Ku, T., Defo, B.K., Kumar, G.A., Larson, H.J., Leinsalu, M., Liang, X., Lim, S.S., Liu, P., Lopez, A.D., Lozano, R., Majeed, A., Malekzadeh, R., Malta, D.C., Mazidi, M., McAlinden, C., McGarvey, S.T., Mengistu, D.T., Mensah, G.A., Mensink, G.B.M., Mezgebe, H.B., Mirrakhimov, E.M., Mueller, U.O., Noubiap, J.J., Obermeyer, C.M., Ogbo, F.A., Owolabi, M.O., Patton, G.C., Pourmalek, F., Qorbani, M., Rafay, A., Rai, R.K., Ranabhat, C.L., Reinig, N., Safiri, S., Salomon, J.A., Sanabria, J.R., Santos, I.S., Sartorius, B., Sawhney, M., Schmidhuber, J., Schutte, A.E., Schmidt, M.I., Sepanlou, S.G., Shamsizadeh, M., Sheikhbahaei, S., Shin, M.J., Shiri, R., Shiue, I., Roba, H.S., Silva, D.A.S., Silverberg, J.I., Singh, J.A., Stranges, S., Swaminathan, S., Tabarés-Seisdedos, R., Tadese, F., Tedla, B.A., Tegegne, B.S., Terkawi, A.S., Thakur, J.S., Tonelli, M., Topor-Madry, R., Tyrovolas, S., Ukwaja, K.N., Uthman, O.A., Vaezghasemi, M., Vasankari, T., Vlassov, V.V., Vollset, S.E., Weiderpass, E., Werdecker, A., Wesana, J., Westerman, R., Yano, Y., Yonemoto, N., Yonga, G., Zaidi, Z., Zenebe, Z.M., Zipkin, B. and Murray, C.J.L. (2017): Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N. Engl. J. Med., **377**, 13-27.

Turck, D., Bresson, J.L., Burlingame, B., Dean, T., Fairweather-Tait, S., Heinonen, M., Hirsch-Ernst, K.I., Mangelsdorf, I., McArdle, H.J., Naska, A., Nowicka, G., Pentieva, K., Sanz, Y., Siani, A., Sjodin, A., Stern, M., Tome, D., Loveren, H.V., Vinceti, M., Willatts, P., Lamberg-Allardt, C., Przyrembel, H., Tetens, I., Rakic, J.G., Ioannidou, S., Sesmaisons-Lecarre, A.D., Forss, A.C. and Neuhauser-Berthold, M. (2016): Dietary reference values for thiamin. EFSA Journal., **14**, 1-53.

Yip, R.G. and Wolfe, M.M. (2000): GIP biology and fat metabolism. Life Sci., **66**, 91-103.