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Impact of taurine depletion on glucose control and insulin secretion in mice

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ABSTRACT

Taurine, an endogenous sulfur-containing amino acid, is found in millimolar concentrations in mammalian tissue, and its tissue content is altered by diet, disease and aging. The effectiveness of taurine administration against obesity and its related diseases, including type 2 diabetes, has been well documented. However, the impact of taurine depletion on glucose metabolism and fat deposition has not been elucidated. In this study, we investigated the effect of taurine depletion (in the taurine transporter (TauT) knockout mouse model) on blood glucose control and high fat diet-induced obesity. TauT-knockout (TauTKO) mice exhibited lower body weight and abdominal fat mass when maintained on normal chow than wild-type (WT) mice. Blood glucose disposal after an intraperitoneal glucose injection was faster in TauTKO mice than in WT mice despite lower serum insulin levels. Islet beta-cells (insulin positive area) were also decreased in TauTKO mice compared to WT mice. Meanwhile, overnutrition by high fat (60% fat)-diet could lead to obesity in TauTKO mice despite lower body weight under normal chow diet condition, indicating nutrition in normal diet is not enough for TauTKO mice to maintain body weight comparable to WT mice. In conclusion, taurine depletion causes enhanced glucose disposal despite lowering insulin levels and lower body weight, implying deterioration in tissue energy metabolism.

1. Introduction

Taurine (2-aminoethanesulfonic acid) is widely distributed in nature and in mammals is present in millimolar concentrations in most tissues. Taurine is clinically approved in Japan for the treatment of patients with both chronic heart failure and hepatic disorders (1,2). Moreover, evidence from human clinical and animal studies suggests a beneficial effect of taurine against a variety of other diseases, including diabetes and obesity (3,4). Human taurine content is derived from its biosynthesis in liver, fat, brain etc. and from dietary intake of meat. Seafood is especially rich in taurine (5–7). Tissue taurine content is influenced by diet, as well as by disease and aging (8–11). Dietary taurine insufficiency causes a decrease in plasma taurine levels, which leads to various disorders, such as retinal degeneration, dilated cardiomyopathy and the reduction in reproductive performance in cats which have a low synthetic capacity for taurine (12–14). Importantly, urinary taurine excretion, a marker of taurine intake from the diet, is inversely correlated with mortality rate caused by ischemic heart disease in humans (15,16), indicating the nutritional importance of taurine to prevent lifestyle-related diseases. Moreover, it has been demonstrated that taurine supplementation attenuates obesity, diabetes and hypercholesterolemia in diet-induced and inherent obesity experimental models (17–20).

The taurine transporter (TauT), which transports taurine from the extracellular space into cells to help maintain a high intracellular taurine content, is widely expressed in various tissues. The taurine transporter knockout (TauTKO) mouse exhibits extensive taurine depletion in several tissues (21,22). Especially noteworthy is the 98% decrease in taurine content in heart and skeletal muscle of the TauTKO mice, compared to about 1–2% in WT mice. By comparison, taurine levels in other tissues of the TauTKO mouse, such as brain, kidney and liver, falls to 10–30% of those found in WT mice, indicating a very low capacity of taurine biosynthesis in the heart

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and skeletal muscle. TauTKO mice exhibit lower body weight than WT mice although the amount of food intake is identical (21–23). TauTKO mice also exhibit decreased skeletal muscle weight and cell size, indicating that muscle atrophy contributes to body weight loss. Endurance running time of TauTKO mice is lower than that of the control mice. Moreover, blood glucose is cleared faster during treadmill running in TauTKO mice than in WT mice (23), which may contribute to a reduction in exercise capacity. Taurine depletion alters the respiratory quotient during exercise (21,24), implying that taurine depletion affects the balance in energy metabolism. Taurine has been recently implicated in the regulation of mitochondrial function through various actions, such as modulation of mitochondrial transfer RNA, buffer action and calcium movement (25–27), supporting the idea that taurine might play an important role in energy production. Additionally, several lines of evidence reveal a crucial role of taurine in β cell function. It has been reported that taurine treatment attenuates cell injury induced by several stresses in the islets (28–30). Moreover, long-term taurine supplementation of mice fed a normal diet of taurine reduces plasma glucose during a glucose tolerance test concomitant with an increase in islet size of the pancreas. Therefore, it is logical to assume that taurine depletion may affect the regulation of blood glucose.

In this study, we investigated the effect of taurine depletion using TauTKO mice on blood glucose control, insulin secretion and high fat diet-induced obesity.

2. Materials and methods

2.1. Animal care

The experimental procedures were approved by the Institutional Animal Care and Use Committee of Hyogo University of Health Sciences. TauTKO and littermate mice (C57BL/6 background) were housed in a SPF environment, fed standard chow (MF, Oriental Yeast, Tokyo), had access to water ad libitum and maintained on a 12-h light/dark cycle. Male WT and TauTKO mice were studied.

2.2. High fat diet

In the high fat diet group, mice were fed 60% fat-contained diet (Oriental Yeast) beginning at 3 months of age. Body weight was monitored each week. Sixteen weeks after HFD, mice were subjected intraperitoneal glucose tolerance test. All mice were sacrificed 20–22 weeks after initiation of the high fat diet and tissues were quickly frozen in liquid nitrogen and stored at −80 °C.

2.3. Glucose and insulin tolerance tests

For the intraperitoneal glucose tolerance test, after an overnight fast (16–17 h), mice were injected i.p. a glucose solution (1 g/10 mL water) at 1 mg/g body weight. In the insulin tolerance test, normally fed mice were fasted for 3 h, and then administered i.p. insulin (0.2 U/g body wt) (Nacalai Tesque, Kyoto, Kyoto). Blood glucose levels before and 15, 30, 60, 90 and 120 min after injection were measured in tail vein blood using Precision Xceed with Blood Glucose Test Strips (Abbott Japan, Tokyo). Serum insulin levels before and 15 and 60 min after glucose injection was determined by using an insulin kit (Morinaga Institute of Biological Science, Yokohama, Kanagawa) according to manufacturer's protocol.

2.4. Measurement of tissue taurine content by HPLC

Tissues were homogenized in 100 mM HEPES (pH 7.5). Four volume of 5% sulfosalicylic acid was added to the tissue lysate. After centrifugation, the supernatant was filtered and neutralized with 1 M $NaHCO_3$. Then, samples were subjected to HPLC to determine taurine concentration, using a previous method with slight modification (31). In brief, supernatant was derivatized with an OPA reagent (3 mg of *o*-phthalaldehyde with 50 μL of 95% ethanol, 10 μl of 2-mercaptoethanol in 5 mL of 100 mM borate buffer (pH 10.4)), and then applied to HPLC (D-2000, Hitachi High Technologies, Tokyo) equipped with a reverse phase column (Cosmosil 5C18-MS-II, 150 mm, Nacalai Tesque).

2.5. Westernblot

For preparation of the membrane fraction of skeletal muscle and liver, tissues were minced and homogenized in isotonic buffer (100 mM Sucrose, 100 mM Tris, 45 mM KCl, 10 mM EDTA, pH 7.4), and then were centrifuged to remove debris, nuclei and the mitochondrial fraction. Supernatant was obtained after centrifugation at 200,000× *g* for 1 h (MLA-50, Beckman Coulter, Miami, FL, USA), with the pellet defined as the membrane fraction. The membrane pellet was dissolved in RIPA buffer.

After protein determination using the bicinconic acid assay method (Pierce BCA assay kit, Life Technologies, Grand Island, NY, USA), protein samples were subjected to western blots as previously described (32). Anti-glucose transporter (Glut) −1, −2, −4 (Millipore, Billerica, MA, USA; 1:500) antibodies were used as 1st antibodies.

2.6. Histological analysis

Sections from frozen tissues were cut by cryostat (Carl Zeiss, Jena, Germany). Sections we stained by hematoxylin & eosin methods. For detection of insulin positive cells, pancreatic sections were immunostained by using anti-insulin antibody (ab80, Abcam, Cambridge, UK; 1:100) and Alexa Fluor 488-conjugated second antibody (Life Technologies; 1:400) with Can Get Signal Immunostain according to manufacturer's protocol (Toyobo, Osaka, Osaka). Images were acquired with microscopes (BZ-9000, Keyence, Osaka, Osaka) equipped with imaging software (BZ-II, Keyence).

2.7. Statistics

Each value was expressed as the mean ± standard error (SE). Statistical analysis was performed using Statcel 2nd edition (OMS Publishing Inc). Analysis of variance (ANOVA) was used to analyze blood glucose and body weight changes. Student's t-test or Tukey–Kramer test was used to determine statistical significance between groups. Differences were considered statistically significant when the calculated p value was less than 0.05.

3. Results

3.1. Lean phenotype in TauTKO mice

When mice were fed normal chow, the body weight of TauTKO mice was lower than that of the WT mice at both 3 months and 12 months of age (Fig. 1A) (genotype, $F = 27.66$, $p < 0.001$; time, $F = 55.15$, $p < 0.001$; interaction, $F = 3.64$, $p > 0.05$ by repeated measures two-way ANOVA, $p < 0.01$ between 3-month-old WT and TauTKO, $p < 0.01$ between 12-month-old WT and TauTKO by Tukey–Kramer test) (22). While WT mice contained visceral adipose tissue at 1 year of age, visceral fat deposition was less obvious in TauTKO mice, as the weight of visceral fat was significantly less in TauTKO mice at 1 year of age than in their corresponding WT cohorts ($p < 0.01$ by Student's t-test) (Fig. 1B and C). Dietary intake was not different between WT and TauTKO mice. These data