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Intake of mulberry 1-deoxynojirimycin prevents diet-induced obesity through increases in adiponectin in mice

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ABSTRACT

In this study, the anti-obesity effect of 1-deoxynojirimycin (DNJ) was examined in the diet-induced obese mouse model. Mulberry DNJ was administered to the obese mice for 12 weeks. As a result, DNJ decreased both the visceral fat weight and adipocyte size. To determine the influence of DNJ on lipid metabolism, lipid parameters of the plasma and the liver and the activities of several molecules related to lipid metabolism in the liver were measured. DNJ activated the β-oxidation system, suppressed lipid accumulation in the liver and reduced plasma triacylglycerol. Since it was thought that the factor activated in the β-oxidation system was adiponectin, plasma adiponectin levels were measured and it was shown that plasma adiponectin was increased with DNJ. Therefore, it was suggested that DNJ promoted an increase in plasma adiponectin and activated the β-oxidation system. Overall, it was shown that DNJ prevents diet-induced obesity through an increase in adiponectin.

1. Introduction

1-Deoxynojirimycin (DNJ) is a D-glucose analogue, in which the oxygen atom of the pyranose ring is substituted by an NH group. DNJ is a characteristic constituent of mulberry (Moraceae) leaves, and dietary mulberry DNJ may be beneficial for suppression of abnormally high blood glucose levels (Asano et al., 2001; Singab, El-beshbishy, Yonekawa, Nomurac, & Fukai 2005). To test this hypothesis, we previously conducted a study of mulberry DNJ in humans and showed a suppressive effect on the postprandial increase in blood glucose (Kimura et al., 2007). This suggests that mulberry DNJ could be feasible for oral treatment of non-insulin-dependent diabetes mellitus (type 2 diabetes). While these findings are of interest, the other physiological functions of DNJ remain unknown. We also recently examined the influence of oral administration of DNJ mulberry extracts on lipid metabolism in normal rats (Tsuduki et al., 2009). Intake of DNJ mulberry extracts suppressed lipid accumulation in the liver. From a safety perspective, DNJ mulberry extracts decreased oxidative stress and did not induce liver dysfunction. Therefore, it was thought that suppression of lipid accumulation into the body was a new physiological action of DNJ. In other words, DNJ would be expected to have an anti-obesity effect.

Total caloric intake is increased by excessive intake of lipids and can lead to obesity (Bray & Popkin, 1998). Obesity is characterised by an increase in adipose tissue and is the basis of lifestyle diseases. Lifestyle diseases are also referred to as diseases of longevity or civilization, and include diabetes mellitus, hyperlipidemia and arteriosclerosis (Nakamura et al., 1994). Research over the past decade has shown that adipose tissue is not used merely for energy storage, but also has an important endocrine function in secreting an array of proteins known as adipokines, including adiponectin and leptin (Hu, Liang, & Spiegelman, 1996). Adiponectin improves insulin sensitivity and decreases plasma glucose and free fatty acid (FFA) levels, while increasing fatty acid oxidation in liver and muscle (Yamauchi et al., 2001). These properties suggest that adiponectin may have therapeutic effects against lifestyle diseases and obesity. Therefore, health food supplements and medicines that enhance plasma adiponectin levels are of interest. In addition, we recently showed that DNJ mulberry extracts decreased oxidative stress and lipid peroxidation in normal rats (Tsuduki et al., 2009). Since lipid peroxidation increased in obese subjects and can lead to adverse effects (Furukawa et al., 2004), it is possible that DNJ would improve the adverse effects caused by obesity if DNJ can prevent lipid peroxidation.

In this study, the anti-obesity effect of DNJ was examined using the diet-induced obese mouse model. Mulberry DNJ was administered to obese mice for 12 weeks. The visceral fat weight and

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adipocyte size were measured to evaluate the obesity symptoms in the mice. To investigate the influence of DNJ on lipid metabolism, lipid parameters in both plasma and the liver and the activities of some molecules related to lipid metabolism in the liver were measured. Moreover, to evaluate the safety of DNJ, indices of hepatic dysfunction and lipid peroxidation were measured.

2. Materials and methods

2.1. Materials

DNJ was extracted from mulberry leaves (*Morus alba*) and purified using ion-exchange chromatography followed by recrystallization, as described previously (Kimura et al., 2007; Nakagawa et al., 2007). The purity of DNJ was shown to be >98% by hydrophilic interaction liquid chromatography with hybrid quadrupole/linear ion trap tandem mass spectrometry (HILIC-QTRAP MS/MS), as described previously (Nakagawa et al., 2008). NaCl, 10% formalin, sucrose and Tris–HCl were purchased from Wako Pure Chemicals Industries (Osaka, Japan). Acetyl CoA, 5,5′-dithio-bio-(2-nitrobenzoic acid) (DTNB), malonyl CoA, NADPH, palmitoyl CoA and L-carnitine were purchased from Sigma (St. Louis, MO). EDTA was purchased from Dojindo (Kumamoto, Japan).

2.2. Animals and diets

In this study, all procedures were performed in accordance with the Animal Experiment Guidelines of Tohoku University. The animal protocol was approved by the Animal Use Committee at Tohoku University. Male C57BL/6 J mice (3 weeks of age) were obtained from Japan Clea (Tokyo, Japan). To induce obesity, a high-fat diet was provided to the mice. MF (a control diet) used for animal trials was purchased from Oriental Yeast Co. (Chiba, Japan). The High Fat Diet 32 (a high-fat diet) used for animal trials was purchased from Japan Clea. The diet composition (control diet vs. high-fat diet, g/kg diet) included nitrogen-free extract, 540 vs. 294, respectively; crude protein, 238 vs. 255, respectively; crude fat, 51 vs. 320, respectively; crude ash, 32 vs. 40, respectively; crude fibre, 61 vs. 29, respectively; and moisture, 78 vs. 62, respectively. The energy (kcal/100 g diet) was 357 and 508 for the control and high-fat diets, respectively. After acclimatisation to the control diet for one week, 40 mice were randomly divided into 4 groups [control (−) ($n = 8$), control (+) ($n = 8$), DNJ 1 mg/kg body weight (DNJ1, $n = 8$), and DNJ 5 mg/kg body weight (DNJ5, $n = 8$)]. The control diet was provided *ad libitum* to the control (−) group for 12 weeks. The high-fat diet was provided *ad libitum* to the control (+), DNJ1 (1 mg DNJ/kg body weight/day), or DNJ5 (5 mg DNJ/kg body weight/day) groups for 12 weeks. The dosage of DNJ (1 mg/kg body weight/day) exhibited an anti-fatty liver effect in our recent study (Tsuduki et al., 2009). To further clarify the effect of DNJ, mice were also treated with a high dose of DNJ (5 mg/kg body weight/day). DNJ dissolved in 0.9% NaCl (w/w) or 0.9% NaCl only were administered orally by direct stomach intubation for 12 weeks. Mice were housed four per cage and had free access to the respective diets and distilled water in a temperature- and humidity-controlled room with light cycles of 12 h on and 12 h off, as described previously (Honma et al., 2012; Shinohara et al., 2012). At the end of the 12-week period, the mice were weighed and blood samples were collected by decapitation. The liver, perirenal adipose tissue, mesenteric adipose tissue, and epididymal adipose tissue were removed and weighed. Blood was treated with EDTA and plasma was isolated by cold centrifugation at 1000×*g* for 15 min at 4 °C (CAX-370 Hybrid Refrigerated Centrifuges, Tomy Digital Biology, Tokyo, Japan), as reported previously. Livers, adipose tissues and plasma were stored at −80 °C until use.

2.3. Histological analysis of the liver and adipose tissue

The liver and adipose tissue were fixed in 10% formalin and embedded in paraffin, as described previously (Honma et al., 2012; Takasaki et al., 2012). Vertical sections (5 μm) were cut, mounted on a glass slide, stained with hematoxylin and eosin, and observed using a microscope (BZ-9000; Keyence, Osaka, Japan). The adipocyte size was calculated by counting the number of cells in a constant view that was selected at random (10 times per mouse).

2.4. Biochemical analyses of plasma and liver

Biochemical analyses of plasma and liver samples were performed as described previously (Honma, Yanaka, Tsuduki, & Ikeda, 2011; Tsuduki et al., 2009). Triacylglycerol (TG) and total cholesterol (TC) levels in plasma and liver, and Alanine aminotransferase (ALT), aspartate aminotransferase (AST), FFA, and glucose levels in plasma were measured using commercial enzyme kits (Wako Pure Chemical, Osaka, Japan), according to the manufacturer's instructions. The phospholipid (PL) content in plasma and liver was determined according to the method described by Bartlett (Bartlett, 1957; Tsuzuki, Tokuyama, Igarashi, & Miyazawa, 2004). Insulin and adiponectin levels in plasma were determined using ELISA kits (Shibayagi, Shibukawa, Japan), according to the manufacturer's instructions.

2.5. Enzyme activity analyses

The activities of hepatic enzymes [fatty acid synthase (FAS, EC 2.3.1.85), malic enzyme (ME, EC 1.1.1.39), carnitine palmitoyltransferase (CPT, EC 2.3.1.21) and acyl-CoA oxidase (ACO, EC 1.3.3.6)] were measured, as described previously (Tsuzuki, Kawakami, Nakagawa, & Miyazawa, 2006). Livers were homogenised with 0.25 mol/L sucrose containing 1 mmol/L EDTA and 10 mmol/L Tris–HCl buffer (pH 7.4), and then centrifuged at 700×*g* for 10 min (CAX-370 Hybrid Refrigerated Centrifuges, Tomy Digital Biology, Tokyo, Japan) to eliminate nuclei and obtain the whole liver homogenates. The supernatant fraction was centrifuged at 125,000×*g* for 60 min (Optima MAX Ultracentrifuge, Beckman Coulter, Brea, USA) to obtain the cytosolic fraction. The whole liver homogenate was used to measure the activity of CPT and ACO, while the cytosolic fractions were used to measure the FAS and ME activity. All enzyme activities were measured spectrophotometrically (Infinit F200; Tecan Japan, Kawasaki, Japan). The FAS activity was measured by the reduction of NADPH, using acetyl CoA and malonyl CoA as substrates. The ME activity was measured by the increase of NADPH, using malic acid as substrate. The CPT activity was determined by the reaction of DTNB with CoA released after the addition of palmitoyl CoA and L-carnitine. The ACO activity was estimated as the level of hydrogen peroxide produced during the oxidation of palmitoyl CoA after the addition of peroxidase.

2.6. mRNA expression analysis

For real-time quantitative reverse transcriptase PCR (qRT-PCR), total RNA was isolated from liver using the RNeasy Mini Kit (Qiagen, Valencia, CA) (Tsuzuki et al., 2007; Tsuzuki & Kawakami, 2008; Yanaka et al., 2011), after elution with 30 μL RNase-free water, and stored at −80 °C until use. To quantify the expression genes, the mRNA levels of acyl-Coenzyme A oxidase (*Aco*), carnitine palmitoyltransferase (*Cpt*), fatty acid synthase (*Fas*), malic enzyme (*Me*), peroxisome proliferator activated receptor alpha (*Ppara*), sterol regulatory element binding factor 1 (*Srebf1*) and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) in liver were determined using a TP870 Thermal Cycler Dice Real Time System