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ARTICLE

Intake of 1-Deoxynojirimycin Suppresses Lipid Accumulation through Activation of the β-Oxidation System in Rat Liver

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It was recently shown that administration of 1-deoxynojirimycin (DNJ) extracted from mulberry suppresses an increase in postprandial blood glucose in humans. These findings are of interest, but other physiological functions of DNJ are unknown. This study examined the effects of oral administration of DNJ (1 mg/kg of body weight/day) or mulberry extracts enriched in DNJ (meDNJ; 100 or 200 mg of extract/kg of body weight/day, equivalent to 0.53 or 1.06 mg of DNJ/kg of body weight/day) in male Sprague−Dawley rats for 4 weeks. DNJ and meDNJ enhanced expression of adiponectin mRNA in white adipose tissue; increased plasma adiponectin levels, enhanced expression of AMPK mRNA, activated the β-oxidation system, and suppressed lipid accumulation in the liver. Intake of DNJ and meDNJ did not cause hepatic dysfunction and led to a reduction of oxidative stress. These results indicate the efficacy and safety of DNJ and meDNJ.

KEYWORDS: Adiponectin; AMPK; β-oxidation; deoxynojirimycin; mulberry; rat

INTRODUCTION

Total caloric intake is increased by excessive intake of lipids, and this leads to the development of obesity (*1*). Obesity is characterized by an increase in adipose tissue and is the basis of lifestyle diseases. These diseases are also referred to as diseases of longevity or civilization and include diabetes mellitus, hyperlipidemia, and arteriosclerosis (*2*, *3*). Research in 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 (*4*, *5*). Adiponectin improves insulin sensitivity and decreases plasma glucose and nonesterified fatty acid levels while increasing fatty acid oxidation in liver and muscle (*6*). These properties suggest that adiponectin may have therapeutic effects against lifestyle diseases and obesity, and medicines and health food supplements that enhance the plasma adiponectin level are therefore of interest.

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 an abnormally high blood glucose level (*7*−*10*). To evaluate this hypothesis, we recently conducted a study of mulberry DNJ in humans and showed a suppressive effect on postprandial blood glucose (*11*). This suggests that use of mulberry DNJ is feasible for oral treatment of non-insulin-dependent diabetes mellitus (type 2 diabetes). These findings are of interest, but other physiological functions of DNJ are unknown. Therefore, in the current study we examined the influence of oral administration of DNJ on lipid metabolism in rat. Mulberry extracts enriched in DNJ (meDNJ) and refined DNJ were administered for 4 weeks to mimic use as a food supplement. Intake of meDNJ and DNJ increased plasma adiponectin, activated the β-oxidation system, and suppressed lipid accumulation in the liver. From a safety perspective, DNJ and meDNJ decreased oxidative stress and did not cause liver dysfunction.

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MATERIALS AND METHODS

Materials. DNJ was extracted from mulberry leaves (*Morus alba*) and purified using ion-exchange chromatography followed by recrystallization, as described previously (*11*, *12*). The purity of DNJ was shown to be >95% by hydrophilic interaction chromatography (HILIC)-MS (*11*). To produce the mulberry extracts enriched with DNJ, we used a procedure that we developed recently (*11*). Mulberry leaves were subjected to hot-air drying, and the dried leaves were broken up and mixed with a mixture of ethanol and water (20:80, v/v). After filtration, the extract was concentrated and lyophilized to a powder. The DNJ content in meDNJ was 0.53% by HILIC-MS.

Animals and Diet. Male Sprague−Dawley rats (4 weeks of age) were obtained from Japan SLC (Hamamatsu, Japan). Commercial diet (MF) used for animal trials was purchased from Oriental Yeast Co. (Chiba, Japan) (*13*). After acclimatization to the commercial diet for 1 week, 32 rats were randomly divided into 4 groups. The 4 groups were control, meDNJ100 (100 mg of extract/kg of body weight/day, equivalent to 0.53 mg of DNJ/kg of body weight/day), meDNJ200 (200 mg of extract/kg of body weight/day, equivalent to 1.06 mg of DNJ/kg of body weight/day), and DNJ (1 mg/kg of body weight/day). DNJ or meDNJ dissolved in 0.9% NaCl (w/w) was administered orally by direct stomach intubation for 4 weeks. Rats were housed with four in each cage and given free access to commercial diet and distilled water in a temperature- and

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humidity-controlled room with light cycles of 12 h on and 12 h off. All procedures were performed in accordance with the Animal Experiment Guidelines of Tohoku University. At the end of the 4 week period, the rats were weighed and blood samples were collected by decapitation. The brain, heart, liver, kidney, 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, as previously reported (*14*). Livers, epididymal adipose tissues, and plasma were stored at −80 °C until use.

Biochemical Analyses in Plasma. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), triacylglycerol (TG), total cholesterol (TC), and glucose in plasma were measured using commercially available enzyme kits (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's protocol. The phospholipid (PL) content in plasma and liver was determined using the method described by Bartlett (*15*, *16*). The insulin level in plasma was determined with a rat/mouse insulin ELISA kit (Linco Research, St. Charles, MO) and the adiponectin level was measured with a rat adiponectin ELISA kit (Chemicon International, Temecula, CA), both according to the manufacturers' protocols.

Enzymatic Activity Analysis. The activities of hepatic enzymes (fatty acid synthase, malic enzyme, carnitine palmitoyltransferase, and acyl CoA oxidase) were measured as described previously (*17*, *18*). Liver was homogenized with 0.25 mol/L sucrose containing 1 mmol/L EDTA and a 3 mmol/L Tris-HCl buffer (pH 7.0) and then centrifuged at 500*g* for 10 min. The supernatant fraction was centrifuged at 9000 × *g* for 10 min to obtain a mitochondrial fraction. The whole liver homogenate (soluble fractions) was used for fatty acid synthase and malic enzyme, whereas the mitochondria fractions were used for carnitine palmitoyltransferase and acyl CoA oxidase. All enzymatic activities were spectrophotometrically measured. The fatty acid synthase activity was measured by the reduction of NADPH, using acetyl CoA and malonyl CoA as substrates. The malic enzyme activities was measured by the increase of NADPH, using malic acid as substrates. The carnitine palmitoyltransferase activity was measured by the reaction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and CoA released after the addition of palmitoyl CoA and L-carnitine. The acyl CoA oxidase activity was estimated as the level of hydrogen peroxide produced during the co-oxidation of palmitoyl CoA when peroxidase was added with palmitoyl CoA.

mRNA Expression Analysis. For the quantitative reverse transcriptase PCR assay, total RNA was extracted from liver and epididymal adipose tissues using a commercial kit (RNeasy Mini Kit, Qiagen, Valencia, CA), as previously described (*19*, *20*). The expression level of acyl-CoA oxidase (ACO), AMP-activated protein kinase (AMPK), carnitine palmitoyltransferase 1 (CPT1), and peroxisome proliferator activated receptor α (PPARα) mRNA in liver and adiponectin mRNA in epididymal adipose tissue were determined with a real-time PCR system (DNA Engine Opticon 2 System, MJ Research, CA), which allows real-time quantitative detection of PCR products by measuring the increase in fluorescence caused by binding of SYBR green to double-stranded DNA. In brief, the cDNA was made using a Ready-To-Go T-Primed First-Strand Kit (Amersham Pharmacia Biotech, Piscataway, NJ) from the total RNA in liver and epididymal adipose tissue. The cDNA was subjected to PCR amplification using a DyNAmo SYBR Green qPCR kit (Finnzymes, Espoo, Finland) and primers for ACO, AMPK, CPT1, PPARα, adiponectin or β-actin (**Table 1**). Real-time PCR was conducted under conditions suitable for the primers, as established previously (*19*, *20*). The β-actin content in each sample was used to normalize the results.

Determination of Lipid Peroxides. To examine the oxidative stress of plasma and tissue lipids possibly caused by DNJ intake, phospholipid hydroperoxides (PLOOH) were determined by chemiluminescence detection−high-performance liquid chromatography (CL-HPLC) as described previously (*16*, *19*), and thiobarbituric acid reactive substances (TBARS) were determined as described previously (*16*, *19*) in the liver and plasma. The CL-HPLC unit consisted of a JASCO HPLC system (Japan Spectroscopic Co., Tokyo, Japan) combined with a CLD-100 chemiluminescence detector (Tohoku Electronic Industries Co., Sendai, Japan) and a JASCO UV detector (UV-970) equipped with a JASCO Finepak SIL NH2-5 column (*n*-propylamine-bound silica column, 5 μmol/L particle size, 250 × 4.6 mm). The mobile phase applied was 2-propanol/methanol/water (67.5:22.5:10, v/v/v), and the flow rate was 1.0 mL/min.

Table 1. Primer Pairs Used for the Quantitative RT-PCR Reaction

target gene	primer	primer sequence (5′−3′)
ACO	forward	CCAATCACGCAATAGTTCTGG
	reverse	CGCTGTATCGTATGGCGAT
adiponectin	forward	AGGTTGGATGGCAGGCATC
	reverse	GGCTCTCCTTTCCTGCCAG
AMPK	forward	TGGCTCTGGGCATCTTTGTAC
	reverse	ACCACACGCCCTTTCTCAT
CPT1	forward	GGATGGCATGTGGGTAAAAG
	reverse	TACTGACACAGGCAGCCAAA
PPARα	forward	TGAACAAAGACGGGATG
	reverse	TCAAACTTGGGTTCCATGAT
β-actin	forward	CCTGTACGCCAACACAGTGC
	reverse	ATACTCCTGCTTGCTGATCC

Statistical Analysis. Results are expressed as means ± SE. Data were analyzed by a one-way ANOVA, this being followed by inspection of all differences by Tukey's honest significant difference test. A difference was considered to be significant at $P < 0.05$.

RESULTS

Effects of DNJ on Growth Parameters. The effects of oral administration of mulberry extracts enriched in DNJ (meDNJ; 100 or 200 mg of extract/kg of body weight/day; equivalent to 0.53 or 1.06 mg of DNJ/kg of body weight/day) and DNJ (1 mg/kg of body weight/day) were examined in male SD rats over a period of 4 weeks. No significant differences in body weight and food intake were found among the four groups at the end of this period (**Table 2**). The weights of epididymal adipose tissue, a visceral white adipose tissue, in the meDNJ200 and DNJ groups were 69 and 77% of that in the control group, respectively, showing a significant reduction in both groups. There were no significant differences in brain, heart, liver, and kidney weights among the four groups (**Table 2**). These results suggest that DNJ prevents expansion of white adipose tissue only and might cause suppression of lipid accumulation.

Effects of DNJ on Lipid and Sugar Metabolism. To examine the effects of DNJ on lipid and sugar metabolism, the levels of TG, TC, PL, insulin, and glucose in plasma and liver were determined (**Table 3**). The liver TG levels in the meDNJ200 and DNJ groups were 77 and 79% of that in the control group, with a significant reduction in both groups. There were no significant differences in liver TC and PL levels among the four groups. The plasma TG, TC, PL, insulin, and glucose levels also showed no significant differences among the four groups. These results suggest that DNJ reduces TG and prevents lipid accumulation in the liver by influencing liver enzymes for fatty acid metabolism.

Effects of DNJ on the Fatty Acid Metabolism System. To examine changes in fatty acid metabolism in the liver, the activities of fatty acid-synthesizing enzymes, such as fatty acid synthetase and malic enzyme, and fatty acid β-oxidation enzymes, such as carnitine palmitoyltransferase (CPT) and acyl-CoA oxidase (ACO), were measured (**Figure 1**). The CPT activities in the meDNJ100, meDNJ200, and DNJ groups were 148, 149, and 155% of that in the control group, with a significant difference from the control for each group. The ACO activities in the meDNJ200 and DNJ groups were 136 and 142% of that in the control group, again with a significant difference from the control for each group. In contrast, the fatty acid synthetase and malic enzyme activities did not differ significantly among the four groups. To confirm these results, mRNA expression levels

Table 2. Effects of Mulberry Extracts Enriched in DNJ (meDNJ) and DNJ on Growth Parameters in Rats[a]

	control	meDNJ100	meDNJ200	DNJ
initial body wt (g)	126 ± 3	126 ± 2	125 ± 2	126 ± 2
final body wt (g)	297 ± 9	303 ± 4	300 ± 7	298 ± 4
food intake (g/day)	20.4 ± 0.5	21.2 ± 0.8	21.8 ± 0.7	21.1 ± 0.8
brain (g/100 g of body wt)	0.62 ± 0.02	0.61 ± 0.01	0.62 ± 0.03	0.65 ± 0.02
heart (g/100 g of body wt)	0.30 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0.30 ± 0.00
liver (g/100 g of body wt)	3.45 ± 0.13	3.35 ± 0.03	3.62 ± 0.05	3.52 ± 0.04
kidney (g/100 g of body wt)	0.64 ± 0.02	0.66 ± 0.01	0.65 ± 0.02	0.64 ± 0.01
epididymal adipose tissue (g/100 g of body wt)	0.87 ± 0.04a	0.74 ± 0.05ab	0.60 ± 0.04b	0.67 ± 0.03b

[a] Values are means ± SE, $n = 8$. Means in a row with different letters are significantly different at $P < 0.05$. meDNJ100, meDNJ (100 mg of extract/kg of body weight/day); meDNJ200, meDNJ (200 mg of extract/kg of body weight/day).

Table 3. Effect of meDNJ and DNJ on Lipid, Insulin, and Glucose Levels in Rat Plasma and Liver[a]

	control	meDNJ100	meDNJ200	DNJ
plasma lipids (mmol/L)				
triacylglycerol	1.55 ± 0.22	1.50 ± 0.08	1.13 ± 0.14	1.27 ± 0.07
total cholesterol	1.74 ± 0.05	1.68 ± 0.09	1.67 ± 0.09	1.53 ± 0.10
phospholipid	2.19 ± 0.04	2.16 ± 0.03	2.06 ± 0.03	2.14 ± 0.07
liver lipids (μmol/g)				
triacylglycerol	50.7 ± 2.7a	45.4 ± 1.2ab	39.1 ± 1.9b	40.1 ± 1.9b
total cholesterol	8.73 ± 0.31	8.69 ± 0.23	8.52 ± 0.09	8.19 ± 0.22
phospholipid	35.2 ± 3.2	29.4 ± 1.9	31.1 ± 1.7	36.2 ± 1.5
plasma insulin (ng/mL)	1.18 ± 0.16	1.05 ± 0.15	0.88 ± 0.10	1.05 ± 0.13
plasma glucose (mg/dL)	104.4 ± 2.5	95.7 ± 3.8	97.0 ± 2.7	93.4 ± 4.2

[a] Values are means ± SE, $n = 8$. Means in a row with different letters are significantly different at $P < 0.05$. meDNJ100, meDNJ (100 mg of extract/kg of body weight/day); meDNJ200, mueDNJ (200 mg of extract/kg of body weight/day).

for β-oxidation enzymes were examined. The CPT1 mRNA expression ratios in the meDNJ100, meDNJ200, and DNJ groups were 148, 155, and 149% of that in the control group, with a significant difference from the control for each group (**Figure 2**). The ACO mRNA expression ratios in the meDNJ200 and DNJ groups were 189 and 207% of that in the control group, again with a significant difference from the control for each group. These results indicate that DNJ promotes the fatty acid β-oxidation system.

The mRNA expression levels for PPARα (activated by the ligand) and AMPK (activated by adiponectin) were also examined, because these proteins regulate the mRNA expression levels of β-oxidation enzymes. AMPK mRNA expression in the meDNJ200 and DNJ groups was 244 and 243% of that in the control group, with a significant difference from the control for each group (**Figure 2**). In contrast, PPARα mRNA expression did not differ significantly among the four groups. To determine the basis for the increase in AMPK mRNA expression, the mRNA expression levels for the antiobesity hormone adiponectin were measured in plasma and white adipose tissue. The adiponectin mRNA levels in white adipose tissue in the meDNJ200 and DNJ groups were 144 and 151% of that in the control group, with a significant difference from the control for each group (**Figure 3**). The adiponectin mRNA levels in white adipose tissue in the meDNJ200 and DNJ groups were 145 and 149% of that in the control group, again showing a significant difference from the control for each group (**Figure 3**). Thus, these results suggest that DNJ enhances expression of adiponectin mRNA in white adipose tissue and increases the plasma adiponectin level, which stimulates expression of AMPK mRNA, activates the β-oxidation system, and suppresses lipid accumulation in the liver.

Effects of DNJ on Hepatic Function and Oxidative Stress. To evaluate the safety of DNJ, indices of hepatic dysfunction (AST and ALT) and oxidative stress (PLOOH and TBARS) were measured (**Table 4**). No significant differences in plasma AST and ALT levels were found among the four groups. The plasma PLOOH levels in the meDNJ200 and DNJ groups were 47 and 54% of that in the control group, respectively, showing a significant reduction in both groups. The plasma TBARS levels did not differ significantly among the four groups. The liver PLOOH levels in the meDNJ200 and DNJ groups were 51 and 53% of that in the control group, respectively, also showing a significant reduction in both groups. The liver TBARS level in the meDNJ200 group was 89% of that in the control group, with a significant difference from the control. These results suggest that DNJ does not cause liver dysfunction and reduces oxidative stress, indicating that it is a safe material.

DISCUSSION

Aza sugars (also referred to as imino sugars) including DNJ are an important class of glycosidase inhibitors that are of interest as potential therapeutic agents (*23*−*25*). Among the imino sugars, miglitol (Glyset) has been approved as a drug (a second-generation α-glucosidase inhibitor) for type 2 diabetes (*26*), and *N*-butyl-DNJ (Zavesca) has been used to treat patients with type 1 Gaucher disease (*27*). Despite the excellent in vitro α-glucosidase inhibitory activity of DNJ, its efficacy in vivo is only moderate (*28*). Therefore, we consider DNJ to be suitable for use as a "functional food" rather than as a drug. For this reason, we recently produced a food-grade mulberry extract enriched with DNJ and conducted a study in which we showed that administration of this extract suppressed the rise of postprandial blood glucose in humans (*11*). In the current study, we examined the physiological basis for DNJ activity and discovered that DNJ suppresses lipid accumulation through activation of the β-oxidation system in rat liver. These results were obtained for a mulberry extract enriched in DNJ and for the refined DNJ active ingredient, on the basis of the potential use of this ingredient in food.

Intake of DNJ and meDNJ strongly suppressed liver TG levels. Because activation of the fatty acid β-oxidation system in the liver suppresses lipid accumulation (*29*, *30*), the activities of hepatic fatty acid β-oxidation enzymes (carnitine palmitoyltransferase and acyl CoA oxidase) were measured. The activities and mRNA expression levels of these enzymes were increased significantly by DNJ and meDNJ. The fatty acid β-oxidation system is also regulated by PPARα and AMPK (*29*, *30*), and therefore the mRNA expression levels were determined for these proteins. These results showed that AMPK mRNA expression was increased by DNJ and meDNJ. Because AMPK is regulated by the

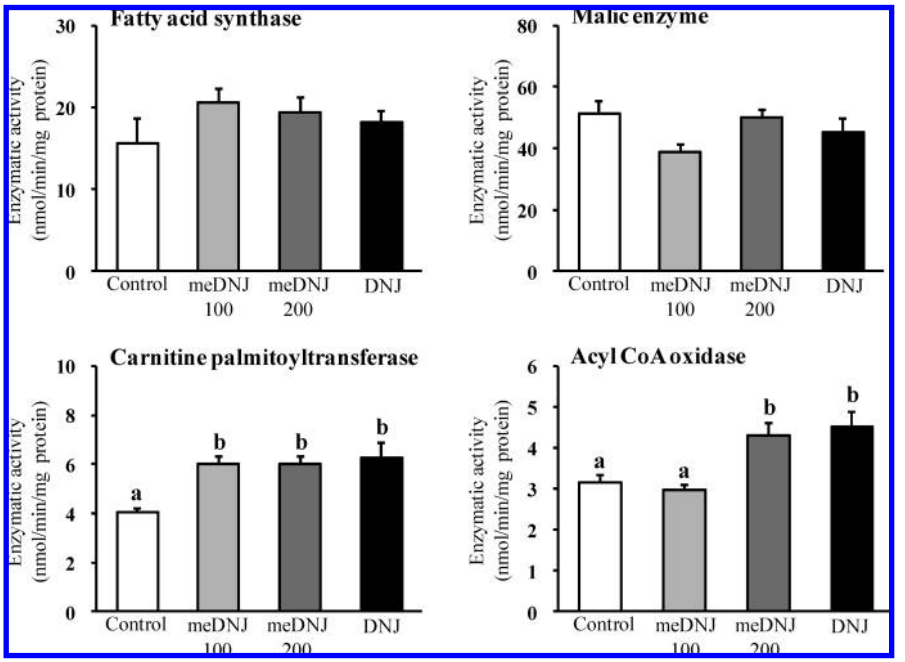

Figure 1. Activity of enzymes in fatty acid metabolism in the liver of rats fed meDNJ and DNJ for 4 weeks. Values are means ± SE, $n = 8$. Different letters above bars indicate significant difference at $P < 0.05$. meDNJ100, meDNJ (100 mg of extract/kg of body weight/day); meDNJ200, meDNJ (200 mg of extract/kg of body weight/day).

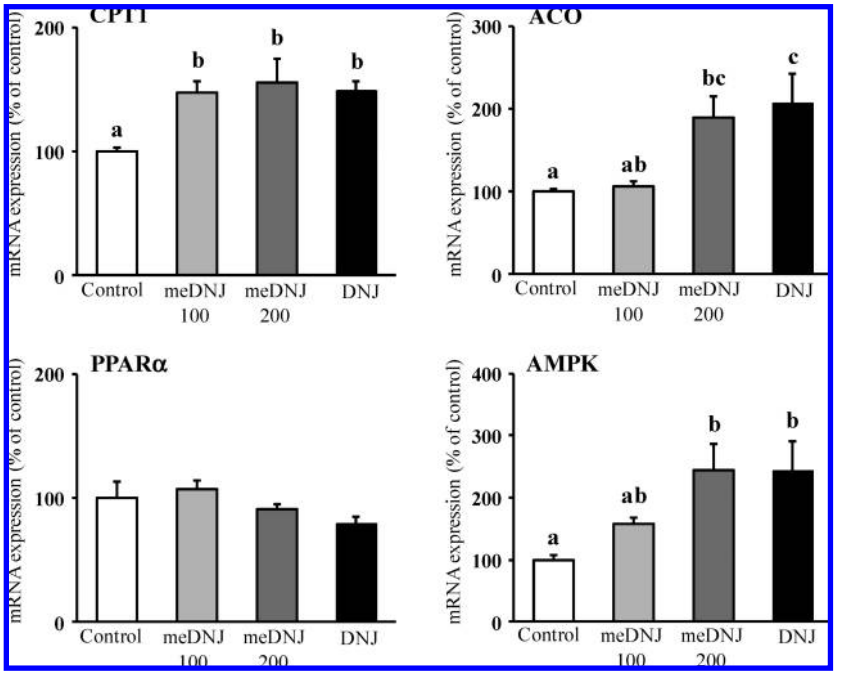

Figure 2. Expression of CPT1, ACO, PPARa, and AMPK mRNA in the liver of rats fed meDNJ and DNJ for 4 weeks. Values are means ± SE, $n = 8$. Different letters above bars indicate significant difference at $P < 0.05$. meDNJ100, meDNJ (100 mg of extract/kg of body weight/day); meDNJ200, meDNJ (200 mg of extract/kg of body weight/day).

antiobesity hormone adiponectin (*29*, *30*), the levels of mRNA expression for adiponectin were measured in plasma and white adipose tissue. These levels were both increased by DNJ and meDNJ. Collectively, these results suggest that DNJ enhances expression of adiponectin mRNA in white adipose tissue and increases the plasma adiponectin level. In turn, this enhances expression of AMPK mRNA, activates the fatty acid β-oxidation system, and suppresses lipid accumulation in the liver.

The weight of epididymal adipose tissue, a visceral white adipose tissue, was decreased by the intake of DNJ and meDNJ. Adiponectin is mainly synthesized and released from white adipose tissue, but paradoxically its level in plasma is reduced in obese human subjects and in patients with metabolic syndrome (*21*, *22*). An increase in plasma adiponectin enhances fatty acid consumption in liver (*6*), consistent with the results in the current study.

There were no significant differences in plasma insulin and glucose levels among the four groups in the study. We have recently shown that administration of mulberry DNJ in humans suppresses an increase in postprandial blood glucose (*11*).

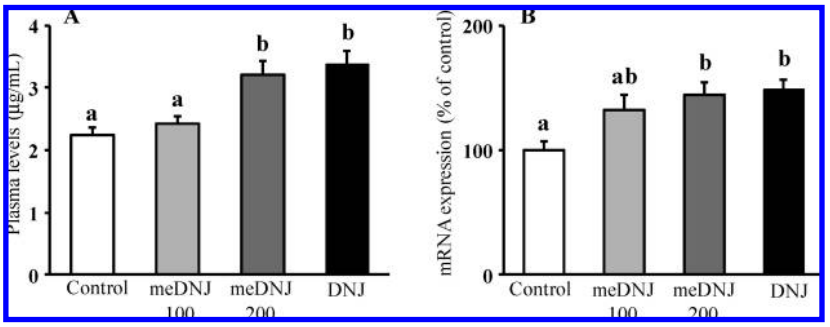

Figure 3. Adiponectin levels in plasma (**A**) and adiponectin mRNA expressions in epididymal adipose tissue (**B**) of rats fed meDNJ and DNJ for 4 weeks. Values are means ± SE, $n = 8$. Different letters above bars indicate significant difference at $P < 0.05$. meDNJ100, meDNJ (100 mg of extract/kg of body weight/day); meDNJ200, meDNJ (200 mg of extract/kg of body weight/day).

Table 4. Effect of meDNJ and DNJ on ALT, AST, PLOOH, and TBARS Levels in Rat Plasma and Liver[a]

	control	meDNJ100	meDNJ200	DNJ
plasma				
AST (IU/L)	47.8 ± 4.1	43.6 ± 3.8	51.3 ± 2.9	54.6 ± 3.4
ALT (IU/L)	21.9 ± 1.3	20.1 ± 1.9	22.4 ± 0.5	20.2 ± 1.4
PLOOH (μmol/mol of PL)	50.7 ± 5.2a	42.5 ± 6.8ab	23.9 ± 3.0b	27.2 ± 3.8b
TBARS (μmol/L)	6.95 ± 1.78	5.13 ± 1.18	3.56 ± 0.91	4.51 ± 0.62
liver				
PLOOH (μmol/mol of PL)	5.21 ± 0.73a	3.25 ± 0.67ab	2.68 ± 0.50b	2.78 ± 0.29b
TBARS (μmol/g of liver)	0.47 ± 0.01a	0.46 ± 0.01ab	0.42 ± 0.02b	0.45 ± 0.01ab

[a] Values are means ± SE, $n = 8$. Means in a row with different letters are significantly different at $P < 0.05$. meDNJ100, meDNJ (100 mg of extract/kg of body weight/day); meDNJ200, meDNJ (200 mg of extract/kg of body weight /day).

However, DNJ did not influence the plasma insulin and glucose levels in rats, which may be because the plasma was collected from fasting normal rats. The plasma insulin and glucose levels in DNJ and meDNJ groups have not changed from those in the control group, and it is likely that DNJ influences these parameters only when these levels are initially abnormal (insulin and glucose levels that are higher than those in the control group). A detailed study in an animal model of obesity and diabetes is needed to examine this hypothesis. The current results indicate that DNJ does not influence sugar metabolism in normal rats.

DNJ and meDNJ intake did not influence indices of liver dysfunction (AST and ALT) and decreased indices of oxidative stress (PLOOH and TBARS) (**Table 4**). Thus, these results suggest that DNJ does not cause liver dysfunction and reduces oxidative stress. These findings indicate that DNJ is a safe material. In addition, we recently showed that ingested DNJ is rapidly eliminated from the body in an intact form, suggesting the absence of side effects (*12*). Hence, a DNJ-enriched extract may be therapeutically useful and safe for the treatment of obesity and non-insulin-dependent diabetes mellitus (type 2 diabetes). Because DNJ suppresses lipid accumulation through activation of the β-oxidation system in rat liver, DNJ may also be useful as a medicine and health food supplement. Further studies are needed to determine the mechanism underlying these effects and to evaluate the effects of DNJ in a disease model.

ABBREVIATIONS USED

ACO, acyl-CoA oxidase; AMPK, AMP-activated protein kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPT, carnitine palmitoyltransferase ; DNJ, 1-deoxynojirimycin; HILIC, hydrophilic interaction chromatography; meDNJ, mulberry extracts enriched with DNJ; PL, phospholipid; PLOOH, phospholipid hydroperoxide; PPARα, peroxisome proliferator activated receptor α; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TG, triacylglycerol.

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