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Antiobesity Effects and Improvement of Insulin Sensitivity by 1-Deoxynojirimycin in Animal Models

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The α-glucosidase inhibitor 1-deoxynojirimycin (DNJ) is one of the simplest naturally occurring carbohydrate mimics, with promising biological activity in vivo. Although there is considerable interest in the pharmacological effects of DNJ, the antidiabetic effects of DNJ in type 2 diabetes mellitus have received little attention. In this work, DNJ was isolated from the silkworm (*Bombyx mori*), and its antidiabetic effects were evaluated in Otsuka Long−Evans Tokushima Fatty (OLETF) rats, an established animal model of human type 2 diabetes mellitus, and in control Long−Evans Tokushima Otsuka (LETO) rats. DNJ treatment showed significant antidiabetic effects in OLETF rats, with significant improvements in fasting blood glucose levels and glucose tolerance and, especially, increased insulin sensitivity. Furthermore, there was significant loss of body weight in both groups. DNJ also showed significant antihyperglycemic effects in streptozotocin- and high-fat-diet-induced hyperglycemic rats. Its efficacy and dose profiles were better than those of acarbose, a typical α-glucosidase inhibitor in clinical use. Furthermore, a substantial fraction of DNJ was absorbed into the bloodstream within a few minutes of oral administration. DNJ was also detected in the urine. These findings suggest that its postprandial hypoglycemic effect in the gastrointestinal tract is a possible but insufficient mechanism of action underlying the antidiabetic effects of DNJ. Its antiobesity effect and improvement of insulin sensitivity are other possible antidiabetic effects of DNJ.

KEYWORDS: 1-Deoxynojirimycin; antidiabetic effect; antiobesity effects; insulin sensitivity

INTRODUCTION

1-Deoxynojirimycin (DNJ) is a potent α-glucosidase inhibitor with low cytotoxicity (*1–4*). Thus, its potential application in the treatment of metabolic disorders, such as diabetes and obesity, has been recognized (*5–7*). Despite its excellent ability to inhibit α-glucosidase in vitro, DNJ has been considered unlikely to become a successful drug. This decision was partly based on its moderate postprandial hypoglycemic efficacy as a single compound in vivo (*8*). Furthermore, DNJ does not inhibit α-amylase, which has prompted the development of new derivatives with altered inhibitory specificities, such as miglitol (BAYm1099) and emiglitate (BAYo1248) (*9*). These derivatives are almost entirely absorbed after oral administration, and the subsequent inhibition of α-glucosidase in the liver may lead to unhealthy storage of glycogen at very high levels (a drug-induced pattern of hepatic glycogen storage mimicking Pompe's disease), because the congenital absence of lysosomal acid 1,4-α-glucosidase activity is the key metabolic defect in glycogen storage disease type II (*10, 11*). Because DNJ was isolated from natural products (*12*), it was suggested that the intake of natural dietary products, such as mulberries or other sericulture products, might be beneficial in suppressing blood glucose levels, thereby preventing diabetes. Recently, several animal and human studies have supported this concept (*5, 8, 9, 13*). Recent reports have considered sericulture products containing DNJ to be suitable for use as functional foods and food additives (*13*). Although there have been many reports of the antihyperglycemic effects of sericulture products and their extracts, there has been no concrete evidence of the antidiabetic effects of DNJ derived from sericulture products as a single compound in an animal model of type 2 diabetes mellitus. Moreover, in a previous study, we found that the DNJ content of commercial sericulture

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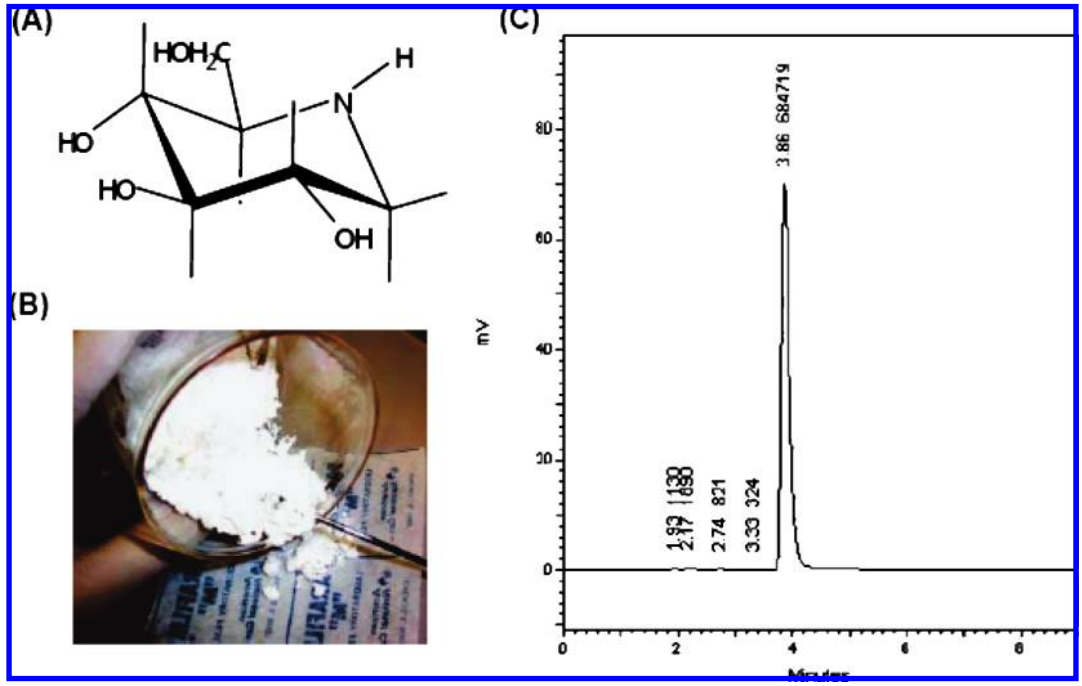

Figure 1. (**A**) Chemical structure of DNJ. The crystallized compound (**B**) was obtained by recrystallization using solvent exchange and analyzed by HPLC (**C**).

products was too low to evaluate the antidiabetic effects of DNJ from sericulture products (<450 mg/100 g of dry silkworm powder). This conclusion is supported by previous studies by Kimura et al. (*13*). Thus, in the present study, we assessed the antidiabetic effects of silkworm (*Bombyx mori*)-derived DNJ in Otsuka Long−Evans Tokushima Fatty (OLETF) rats and their counterpart controls, Long−Evans Tokushima Otsuka (LETO) rats. We also performed a comparative study of DNJ and acarbose in streptozotocin and high-fat-diet (HFD)-induced hyperglycemic (STZ) rats, with several additional tests to evaluate the antidiabetic effects of DNJ.

MATERIALS AND METHODS

Isolation of DNJ. DNJ was isolated by ion exchange chromatography. Briefly, silkworm (Nonghyup, Sanchung-Gun, Korea) extracts were obtained by extraction with 50% methanol, applied to several ion exchange resins and evaporated. Crystallized compounds were obtained by recrystallization using solvent exchange and were analyzed by high-performance liquid chromatography (HPLC; ThermoQuest Co., San Jose, CA) with 9-fluorenylmethyl chloroformate using the method of Kim et al. (*14*). The purity of the isolated DNJ was over 97.0%. **Figure 1** shows the chemical structure of DNJ.

Animals. Male Sprague–Dawley (SD) rats (>200 g; Orient, Seoul, Korea), spontaneously diabetic OLETF rats (15 weeks old), and their counterpart control LETO rats (15 weeks old; Otsuka Pharmaceutical Experimental, Tokushima, Japan) were used. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the U.S. National Institutes of Health and the Animal Experiment Guidelines of Samsung Biomedical Research Institute. All animals were used in the experiments after one week of acclimatization and were killed humanely after the experiments.

Experimental Protocols. *Model of Type 2 Diabetes.* The OLETF and LETO rats were randomly divided on day 0 into two groups (n = 12 each). The animals were treated daily with DNJ (100 mg/kg, dissolved in water, orally) or vehicle (water alone) from 16 weeks of age until the end of the study. Body weights, fasting blood glucose levels, plasma insulin concentrations, and food intake were also measured.

STZ Rat Models. Male SD rats were fed an HFD containing (w/w) 20% fat, 46% carbohydrate, and 20% protein (Glen Forrest Stock Feeders, Glen Forrest, Australia). After two weeks on the HFD, the animals were administered streptozotocin (STZ, 65 mg/kg; Sigma-Aldrich, St. Louis, MO). These STZ- and HFD-induced diabetic rats (STZ rats) (blood glucose > 350 mg/dL) were divided randomly into three groups (n = 10 each) on day 0 and treated with DNJ (20 mg/kg, dissolved in waster, orally), acarbose (20 mg/kg, dissolved in water, orally), or vehicle (water alone) for eight days. The treatments were then discontinued for five days. Blood glucose levels were measured in tail blood samples obtained at fixed times before drug administration.

Intraperitoneal Glucose Tolerance Test (IPGTT). An IPGTT was performed 28 days after each treatment on the OLETF and LETO rats. On test days, the animals were fasted for 14 h and then given an intraperitoneal (ip) injection of glucose (500 mg/kg). Blood glucose levels were measured in tail blood samples at 0, 5, 10, 15, 30, 60, and 120 min after the glucose treatment.

Intravenous Glucose Tolerance Test (IVGTT). An IVGTT was performed 28 days after each treatment on OLETF and LETO rats (n = 8 each group). On the test day, the animals were fasted for 14 h. The animals received glucose (500 mg/kg, dissolved in normal saline, via the jugular vein) or vehicle alone (normal saline) alone. Plasma insulin concentrations were determined from carotid artery blood samples at –30, 0, 1, 3, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after the glucose treatment. An additional IVGTT was performed on normal SD rats (n = 10 each). On the test day, the animals were fasted for 14 h and received continuous infusions (3 mL/kg per hour, via the tail vein) of DNJ (100 μmol/mL, dissolved in normal saline) or vehicle alone (normal saline). Thirty minutes after the infusion, the animals received glucose (500 mg/kg, dissolved in normal saline, via the jugular vein). Blood glucose levels and plasma insulin concentrations were determined from carotid artery blood samples at –30, 0, 1, 3, 5, 10, 15, 30, 60, and 90 min after the glucose treatment.

Blood Glucose Level Determination. Blood glucose levels were determined using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA).

Insulin Measurement. Insulin concentrations in samples were measured by double-antibody radioimmunoassay with rat insulin kits (RI-13K, Linco Research Inc., St. Louis, MO) and a Wallac automatic gamma counter (Perkin-Elmer, Waltham, MA).

Toxicity Test. On day 39, the OLETF and LETO rats were killed by cervical dislocation, and samples of their blood, organs, and tissues were taken to determine blood chemical concentrations and any differences in pathology between groups. Clinical chemistry measure-

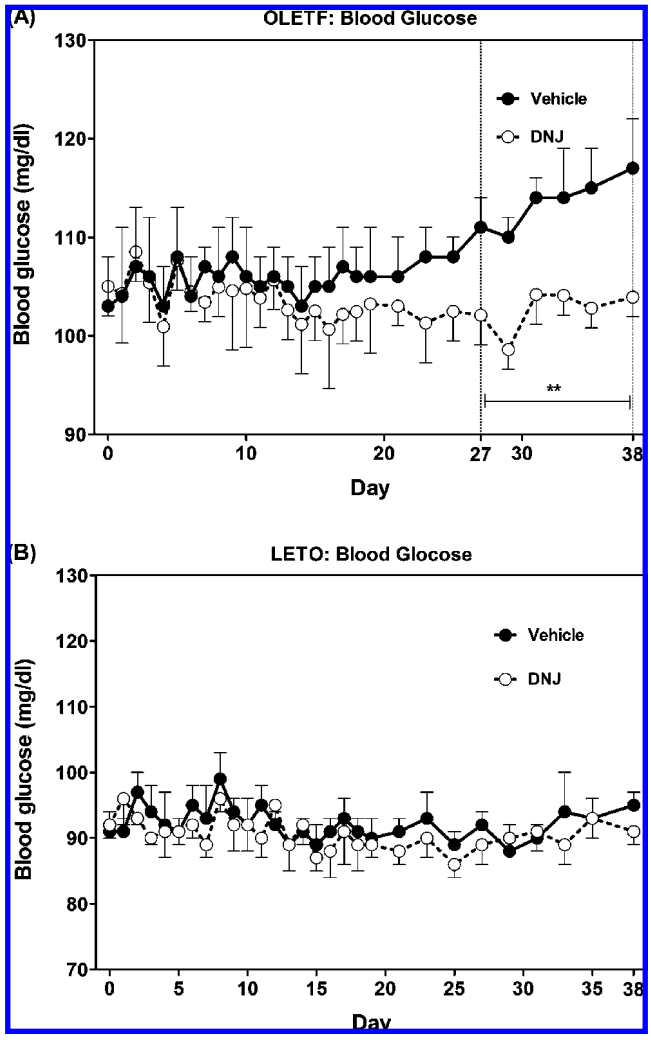

Figure 2. Effects of DNJ on fasting blood glucose concentrations. Fasting blood glucose levels were measured in OLETF (**A**) and LETO (**B**) rats (n = 12 each group) at fixed times (**, $P < 0.01$ vs untreated control rats).

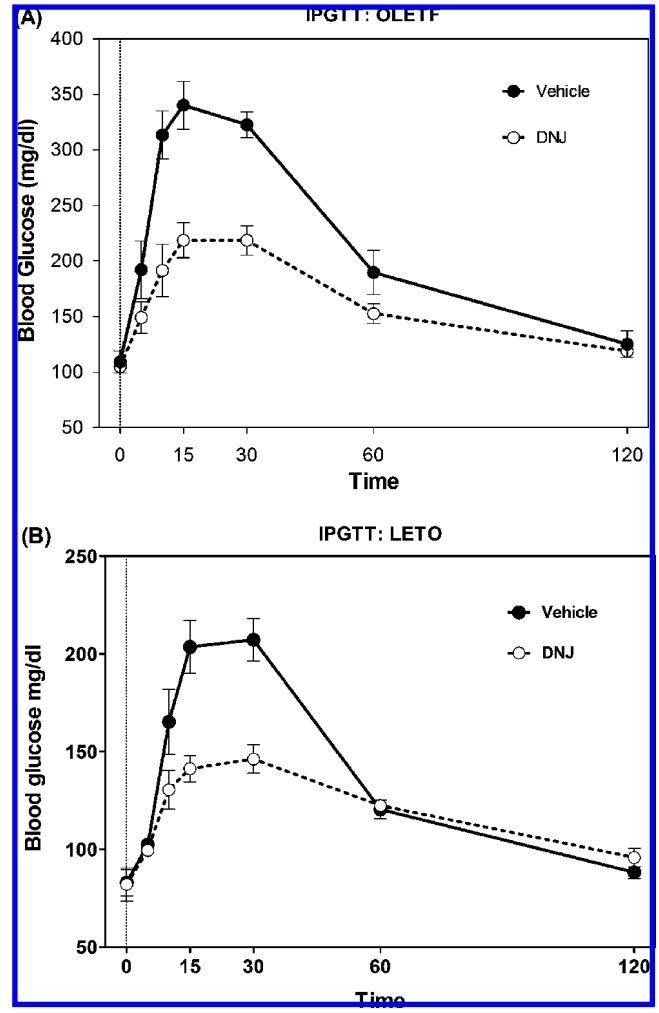

Figure 3. Effects of DNJ on glucose tolerance. An IPGTT was performed in OLETF (**A**) and LETO (**B**) rats (n = 12 each group) on day 28 after medication. On test days, animals were fasted for 14 h and then received glucose (500 mg/kg ip). Blood glucose levels were determined from tail blood samples at 0, 30, 60, and 120 min after glucose treatment.

ments (TG, ALB, T-CHO, TP, GOT, GPT, ALP, BUN, CREA, T-BIL, Ca, and IP), were taken along with hematology measures (Hb, Hct, RBC count, and RBC indices). One-off toxin injections (500, 1000, and 2000 mg/kg, orally) and repetitive toxin injection tests were performed on SD rats (2000 mg/kg per day, orally for two weeks). Bacteria reverse mutation, chromosome aberration, and in vivo micronucleus tests were also performed after treatment with DNJ.

Statistics. Differences between the control and treatment groups were analyzed statistically by one-way ANOVA (Prism, GraphPad; San Diego, CA). All data are expressed as mean ± SEM.

RESULTS

Effects of DNJ in an Animal Model of Type 2 Diabetes Mellitus. *Effects of DNJ Treatment on Blood Glucose Levels.* The fasting blood glucose levels in OLETF and LETO rats were measured daily for 38 days, just before the daily administration of DNJ or vehicle. As shown in **Figure 2**, OLETF rats treated with vehicle alone (**Figure 2A**) underwent a time-dependent increase in fasting glucose levels compared with LETO rats (**Figure 2B**). They also showed significant hyperglycemia after 27 days of treatment ($P < 0.01$). The fasting blood glucose concentrations of LETO rats were not significantly affected in response to treatment with DNJ during the experiment. As shown in **Figure 2A**, in contrast to those of the LETO rats, fasting glucose concentrations decreased steadily and significantly in the OLETF rats in response to DNJ treatment compared with those of the vehicle-treated group (from day 27 to day 38; $P < 0.01$).

Effects of DNJ Treatment on Glucose Tolerance and Plasma Insulin Concentrations. Glucose tolerance in OLETF and LETO rats was evaluated by IPGTT after 28 days of daily medication with DNJ or vehicle. **Figure 3** shows that daily treatment with 100 mg/kg DNJ in OLETF (**Figure 3A**) and LETO rats (**Figure 3B**) significantly affected glucose tolerance compared with vehicle-treated OLETF and LETO rats. The difference in the area under the curve for blood glucose between 0 and 120 min after ip glucose stimulation in DNJ-treated rats was 45% in the OLETF group ($P < 0.01$ vs vehicle-treated OLETF rats) and 27% in the LETO group ($P < 0.05$ vs vehicle-treated LETO rats). The effects of DNJ on plasma insulin concentration were also evaluated by IVGTT after 28 days of daily medication with DNJ or vehicle (**Figure 4**). In contrast to the improvements in blood glucose tolerance, there was a significant reduction in plasma insulin concentrations after glucose stimulation from both OLETF (**Figure 4A**) (from 10 min to 30, 60, and 120 min; $P < 0.05$ vs vehicle-treated OLETF group) and LETO rats (**Figure 4B**) (from 30 to 60 min; $P < 0.05$ vs vehicle-treated LETO group).

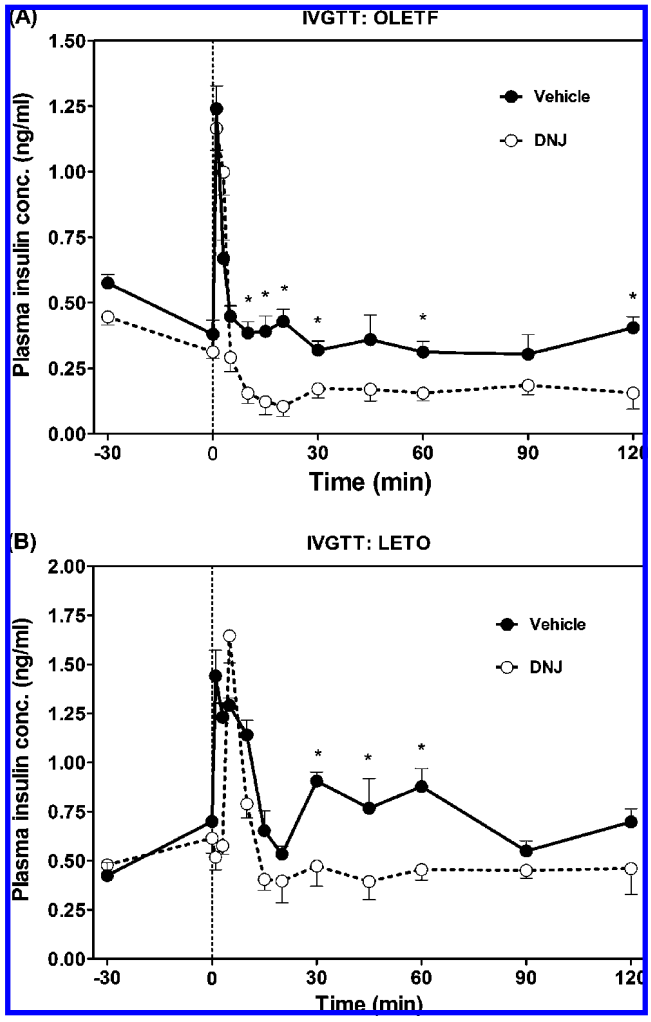

Figure 4. Effect of DNJ on plasma insulin concentrations. An IVGTT was performed on OLETF (**A**) and LETO (**B**) rats. On test days, animals were fasted for 14 h and then received glucose (500 mg/kg). Plasma insulin concentrations were determined from blood samples at –30, 0, 1, 3, 5, 10, 15, 30, 60, 90, and 120 min after glucose treatment (∗, $P < 0.05$ vs untreated control rats).

Effects of DNJ Treatment on Body Weight. **Figure 5** shows the effects of DNJ treatment on body weight changes in OLETF and LETO rats. Weights in the vehicle-treated groups tended to increase during treatment. However, the DNJ-treated animals showed slower weight gains than those of the vehicle-treated controls. Nevertheless, there were no statistically significant effects when compared with those of LETO rats treated with vehicle alone. In the DNJ-treated LETO rats, the mean body weight underwent a time-dependent weight loss. OLETF rats treated with DNJ also underwent a time-dependent weight loss, and there was a significant difference in mean body weights from day 25 to day 28 ($P < 0.01$ vs vehicle treatment).

Effects of DNJ on Glucose Disposal. To clarify the mechanisms of the antihyperglycemic effects of DNJ, we also performed an IVGTT on SD rats after continuous intravenous infusions of DNJ or vehicle alone (**Figure 6**). At 30 min after the start of the infusion, the animals received a 500 mg/kg glucose stimulation. As shown in **Figure 6A**, blood glucose concentrations in SD rats infused with DNJ decreased significantly from 3 to 60 min after glucose stimulation ($P < 0.01$ vs vehicle-treated group). The difference in the area under the curve for blood glucose concentrations between the DNJ-infused animals and the controls was 33% ($P < 0.01$ vs vehicle-treated

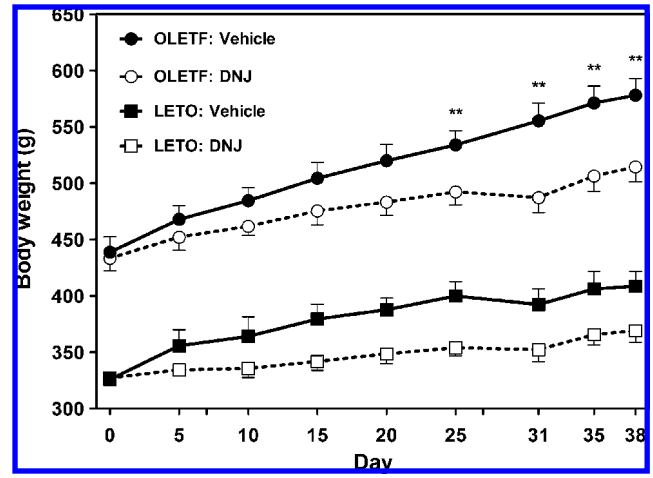

Figure 5. Effect of DNJ treatment on body weight. The OLETF and LETO rats were randomly divided on day 0 into two groups ($n = 12$ each group). Animals were treated with DNJ (100 mg/kg) or vehicle from 16 weeks of age until the end of the study. Body weights were measured at the same time (∗∗, $P < 0.01$ vs untreated control rats).

group). As shown in **Figure 6B**, the plasma insulin concentrations of SD rats infused with DNJ were also significantly reduced ($P < 0.01$ vs vehicle-treated group) from 5 min after glucose stimulation.

Comparative Study. Blood glucose levels were measured in STZ rats for nine days with daily administrations of 20 mg/kg DNJ, 20 mg/kg acarbose, or vehicle alone. The rats were then maintained for five days without treatment. As shown in **Figure 7**, the STZ rats demonstrated significant basal hyperglycemia from day 1 (478 ± 12.4 mg/dL vs normal SD rats 123 ± 12.2 mg/dL; $P < 0.01$). This hyperglycemia was maintained steadily during the entire experiment. Blood glucose concentrations decreased in the DNJ-treated STZ rats from 397 ± 19.3 mg/dL on day 1 to 336 ± 10.5 mg/dL on day 9. In the STZ rats treated with acarbose, blood glucose declined from 437.0 ± 34.5 mg/dL on day 1 to 274 ± 47.3 mg/dL on day 9. There were significant reductions in blood glucose concentrations with both DNJ (50% decrease vs vehicle alone; $P < 0.01$) and with acarbose (49% decrease vs vehicle alone; $P < 0.01$). Blood glucose concentrations decreased steadily from days 2 to 9 in response to acarbose treatment. Unlike acarbose, DNJ did not affect blood glucose concentrations for the first three days. However, blood glucose concentrations decreased significantly from four days after the commencement of treatment (decrease of 47% vs controls; $P < 0.01$) to day 9 (50% decrease vs controls; $P < 0.01$). Blood glucose concentrations in both groups returned steadily to basal hyperglycemia after the treatment was stopped. The DNJ-treated rats showed a slower rebound (257.4 ± 12.6 mg/dL on day 9 to 435.6 ± 12.6 mg/dL on day 14) compared with the acarbose-treated rats (274.3 ± 5.6 mg/dL on day 9 to 486.3 ± 24.1 mg/dL on day 14).

Safety Study. The DNJ medication was well tolerated by the rats, and no specific adverse effects occurred during the in vivo study. There were no significant differences in blood chemistry or pathology between the OLETF and LETO rats (data not shown). Moreover, the results of clinical chemistry, hematology on the SD rats, and fundamental toxicity tests were also unaffected by DNJ treatment (data not shown).

DISCUSSION

The obese hyperglycemic OLETF rat is an established animal model of human type 2 diabetes. It has many similarities to the

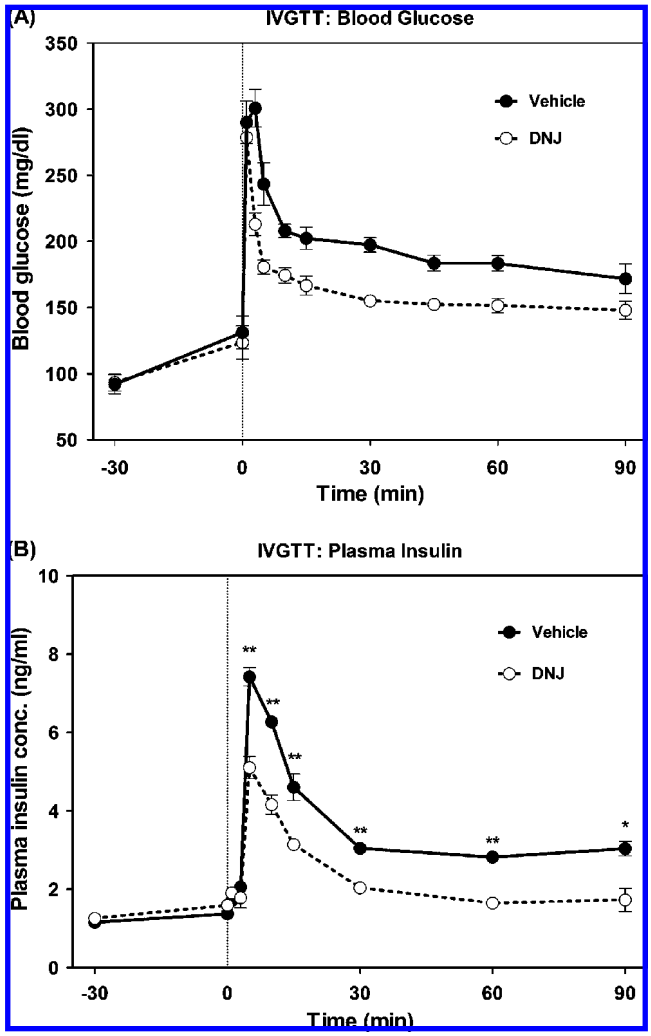

Figure 6. Effect of DNJ on rate of glucose disposal. An IVGTT was performed on normal SD rats. On test days, animals were fasted for 14 h and then received glucose (500 mg/kg) with continuous infusion (3 mL/kg per hour) of DNJ (100 μmol/L) or vehicle ($n = 10$ each). Blood glucose levels (**A**) and plasma insulin concentrations (**B**) were determined from tail blood samples at –30, 0, 1, 3, 5, 10, 15, 30, 60, and 90 min after glucose treatment (∗, $P < 0.05$, and ∗∗, $P < 0.01$ vs untreated control rats).

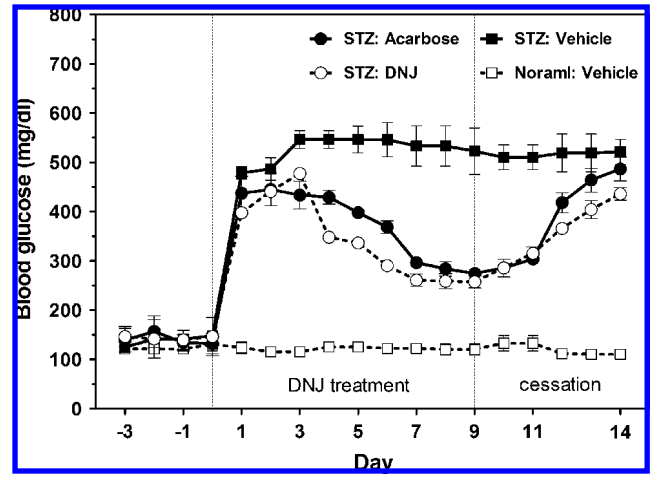

Figure 7. Comparative study using acarbose. STZ rats (blood glucose > 350 mg/dL) were divided randomly on day 0 into three groups ($n = 10$ each) and treated with DNJ (20 mg/kg), acarbose (20 mg/kg), or vehicle alone for 8 days. Treatment was then ceased for 5 days. Blood glucose levels were measured in tail blood samples obtained at fixed times.

human disease, and is characterized by a high degree of insulin resistance compared with that of the control counterpart LETO rat (*15–17*). In our experiment, OLETF rats treated with vehicle alone showed a late onset of chronic and slowly progressive hyperglycemia and innate polyphagia, which caused rapid weight gain and resulted in hyperinsulinemia and insulin resistance. In contrast, DNJ treatment significantly reduced the development of progressive hyperglycemia in OLETF rats. The rate of weight gain was also significantly suppressed, without any significant change in daily food intake. The IPGTT results also showed significant improvements in glucose tolerance in response to DNJ. More importantly, although blood glucose tolerance and fasting blood glucose levels improved, plasma insulin concentrations were significantly reduced followng the IVGTT. This improved glucose tolerance and quantitatively reduced plasma insulin concentrations, clearly indicating an improvement in insulin resistance and/or insulin sensitivity in these rats in response to treatment with DNJ. As in the OLETF rats, glucose tolerance and insulin sensitivity was also significantly improved, and the rate of weight gain was significantly suppressed in the LETO rats. In a comparative study, we used acarbose as the control drug. Acarbose is a competitive inhibitor of small-intestinal brush-border α-glucosidases, developed for therapeutic purposes. Because of its molecular weight and structure, acarbose is apparently not absorbed into the blood (*10, 18*). Parallel to the animal study with OLETF and LETO rats, DNJ treatment also significantly improved hyperglycemia in the STZ rats. Moreover, the efficacy and dose profile of DNJ were better than those of acarbose, showing excellent antihyperglycemic action. Nevertheless, the effective dose of DNJ (about 0.123 mol/kg) was higher than that of acarbose (about 0.031 mol/kg). The efficacy of DNJ was more prolonged than that of acarbose after the cessation of treatment. Interestingly, although DNJ had an excellent antihyperglycemic effect in STZ rats, its significant efficacy was evident from four days after treatment. This shows a significant difference in the efficacy profiles of DNJ and acarbose. As with acarbose, DNJ is generally regarded as a competitive inhibitor of small-intestinal brush-border α-glucosidase. Therefore, the therapeutic consideration of DNJ always centers on its postprandial hypoglycemic effects in the gastrointestinal tract. However, these types of drugs, including acarbose, are known to reduce postprandial blood glucose levels directly by preventing the intestinal absorption of glucose, without directly affecting pancreatic β-cell function, insulin resistance, or fasting glucose concentrations (*18*). Thus, α-glucosidase inhibition within the intestinal tract is a possible mechanism of action of DNJ but is insufficient to explain our results. Furthermore, in a previous study, Nakagawa et al. found that a substantial fraction of DNJ was rapidly absorbed into blood after oral administration (*19*). The inhibition of glucose production from glycogen in the liver by the inhibition of the α-1,6-glucosidase activity of a debranching enzyme is another possible mechanism underlying the antidiabetic effects of absorbed DNJ (*20, 21*). This suggestion is partly based on the finding that the abnormally enhanced hepatic gluconeogenesis caused by the inactivation of the pyruvate dehydrogenase complex is unfavorable in the context of the insulin resistance seen in diabetes (*22*). This suggestion could account for the time lag in the decrease in blood glucose observed in STZ rats treated with DNJ. An antiglycogenolytic action of absorbed DNJ should only be expected when the glucosidase activity of the debranching enzyme is required for

the further phosphorolytic breakdown of glycogen (*20, 21*). Consequently, the antiglycogenolytic activity of DNJ could prevent the progression of insulin resistance in obese rats. In this way, the improved glucose tolerance and fasting glucose concentration resulted in a loss of body weight. Consistent with this suggestion, our IVGTT results showed that an intravenous infusion of DNJ exerted its effects more rapidly than an oral administration. Nevertheless, lysosomal storage of glycogen as a sequela of α-glucosidase inhibition by absorbed DNJ, as discussed above, is a possible adverse effect of absorbed DNJ (*10*). In this situation, the deposition of glycogen will be primarily dependent on the distribution and permeation of the inhibitor. Thus, the dose and lipophilicity of the absorbed compound is a major factor in lysosomal deposition (*10*). More highly lipophilic compounds permeate biological membranes more easily, resulting in their deposition (*10*). This inhibitory activity has also been observed to be dose dependent in vivo (*10*). However, as discussed previously, although DNJ is rapidly absorbed into the blood after oral administration, it then disappears from the body (*19*). This suggestion is supported by other studies (*23, 24*). In particular, Faber et al. (*23*) showed the rapid biphasic plasma disappearance of the DNJ derivative 1-deoxymannojirimycin in vivo after intravenous administration. Moreover, DNJ is a relatively hydrophilic compound when compared with its derivatives, as discussed previously. These facts suggest that the absorbed DNJ may be rapidly eliminated from the body by renal excretion without any adverse effects. Our data indicate that the amelioration of glycogenolytic abnormalities by absorbed DNJ is a possible pharmacological mechanism involved in its antidiabetic effects. Its antiobesity effect and its improvement of insulin sensitivity are other possible antidiabetic effects of DNJ.

In conclusion, we have demonstrated here that treatment with DNJ derived from sericulture products could prevent or delay the development of progressive hyperglycemia, insulin resistance, and rapid weight gain leading to diabetes in animal models, and without any adverse effects. Other antidiabetic effects of DNJ include its antiobesity effect and improvement of insulin sensitivity. Consequently, habitual use of DNJ-enriched products is a feasible therapeutic stratagem for patients with type 2 diabetes mellitus.

ABBREVIATIONS USED

DNJ, 1-deoxynojirimycin; OLETF rats, Otsuka Long−Evans Tokushima fatty rats; LETO rats, Long−Evans Tokushima Otsuka rats; STZ rats, streptozotocin and high-fat-diet-induced hyperglycemic rats; HFD, high-fat diet; HPLC, high-performance liquid chromatography; SD, Sprague–Dawley; IPGTT, intraperitoneal glucose tolerance test; KRBB, Krebs−Ringer bicarbonate buffer; IVGTT, intravenous glucose tolerance test, TG, triglyceride; ALB, albumin; T-CHO, total cholesterol; TP, total protein; GOT, glutamic oxaloacetic transaminase; GPT, guanosine triphosphatase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CREA, creatinine; T-BIL, total bilirubin; Ca, Calcium; IP, inorganic phosphorus, Hb, hemoglobin; Hct, hematocrit; RBC count, red blood cell count; RBC indices, red blood cell indices.

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