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Effects of a nonnutritive sweetener on body adiposity and energy metabolism in mice with diet-induced obesity

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ABSTRACT

Objective. Nonnutritive sweeteners (NNSs) have been studied in terms of their potential roles in type 2 diabetes, obesity, and related metabolic disorders. Several studies have suggested that NNSs have several specific effects on metabolism such as reduced postprandial hyperglycemia and insulin resistance. However, the detailed effects of NNSs on body adiposity and energy metabolism have not been fully elucidated. We investigated the effects of an NNS on energy metabolism in mice with diet-induced obesity (DIO).

Methods. DIO mice were divided into NNS-administered (4% NNS in drinking water), sucrose-administered (33% sucrose in drinking water), and control (normal water) groups. After supplementation for 4 weeks, metabolic parameters, including uncoupling protein (UCP) levels and energy expenditure, were assessed.

Results. Sucrose supplementation increased hyperglycemia, body adiposity, and body weight compared to the NNS-administered and control groups ($P < 0.05$ for each). In addition, NNS supplementation decreased hyperglycemia compared to the sucrose-administered group ($P < 0.05$). Interestingly, NNS supplementation increased body adiposity, which was accompanied by hyperinsulinemia, compared to controls ($P < 0.05$ for each). NNS also increased leptin levels in white adipose tissue and triglyceride levels in tissues compared to controls ($P < 0.05$ for each). Notably, compared to controls, NNS supplementation decreased the UCP1 level in brown adipose tissue and decreased O_2 consumption in the dark phase.

Conclusions. NNSs may be good sugar substitutes for people with hyperglycemia, but appear to influence energy metabolism in DIO mice.

1. Introduction

The consumption of added sugars has increased worldwide [1], and sugar-sweetened foods and beverages can significantly influence the total calorie content and glycemic index of a meal [2]. In addition, excessive intake of high-calorie, high-glycemic-index food can result in exaggerated postprandial glucose levels, potentially leading to metabolic and hormonal

Abbreviations: NNS, nonnutritive sweetener; DIO, diet-induced obesity; BAT, brown adipose tissue; WAT, white adipose tissue; UCP, uncoupling protein; MSL, skeletal muscle; TG, triglyceride; PPAR, peroxisome proliferator-activated receptor; PGC-1, peroxisome proliferator-activated receptor γ coactivator 1; CPT-1, carnitine palmitoyltransferase 1; FAS, fatty acid synthetase; POMC, proopiomelanocortin; NPY, neuropeptide Y.

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changes and promoting body fat deposition. Nonnutritive sweeteners (NNSs) can be a helpful tool for reducing energy intake and body weight, thereby reducing the risk for type 2 diabetes and related metabolic disorders [3–5]. Considering the prevalence of these diseases, NNSs can be an important alternative to natural, calorie-containing sweeteners. However, findings regarding the effects of NNSs on energy intake and body weight have been mixed. Most studies indicate that several NNSs may assist with obesity and/or type 2 diabetes [3,6,7]. Other studies suggest that several NNSs may paradoxically lead to weight gain in different situations [8,9]. For instance, rats that consumed saccharin-sweetened liquids had an increased food intake and gained more body weight than rats that consumed glucose-sweetened liquids [10]. Conversely, individuals that consumed *Stevia*, a natural NNS, had significantly lower postprandial glucose responses than those who consumed sucrose [11]. In addition, rats that consumed sucralose had a significant decrease in beneficial gut bacteria with resultant weight gain [12]. In a study that compared the body's response to sucrose and sucralose, the sucralose did not raise blood sugar levels or increase insulin resistance [13]. Findings regarding the influence of NNSs on body adiposity in both controlled intervention trials and prospective observational studies have been inconsistent.

Adipose tissue, which is classified into brown adipose tissue (BAT) and white adipose tissue (WAT), is the main endogenous source of circulating lipids as well as the site of production and secretion of several hormones and cytokines, including the adipocytokine leptin [14,15]. Studies have demonstrated that leptin plays key roles in a complex network that appears to modulate obesity and related metabolic disorders, including insulin resistance. Uncoupling protein (UCP) 1 in BAT has a role in energy expenditure in both humans and rodents [16–18]. UCP2 is expressed ubiquitously in peripheral tissues, including WAT [18–21], while UCP3 is expressed mainly in skeletal muscle (MSL) and adipose tissues [21,22]. Levels of these proteins are regulated by several humoral factors and environmental temperature [17,22]. Leptin and UCPs can be considered indicators of energy metabolism [23–25].

The present study investigated the effects of preloads containing an NNS or sucrose on food intake, body adiposity, energy metabolism, and leptin and UCP levels in mice with obesity induced by a diet of 60% fat.

2. Methods

2.1. Animals

Mature male mice (C57Bl/6; KBT Oriental, Fukuoka, Japan) (n = 5 per group) were housed in a light-, temperature-, and humidity-controlled room (12-h light/12-h dark cycle, lights on/off at 07:00/19:00 h; 21 ± 1 °C; 55% ± 5% relative humidity). The mice were allowed free access to 60% high-fat food (cat. no. D12492: 20% protein, 20% carbohydrate, 60% fat; 5.2 kcal/g; Research Diets, Tokyo, Japan) and water. The high-fat food contained soybean oil (25/773.85 g) and lard (245/773.85 g). All animals were treated in accordance with the Oita University Guidelines for the Care and Use of Laboratory Animals.

2.2. Measuring food intake and body weight

High-fat diets were administered for 4 weeks to mice (from 8 to 12 weeks of age). The obese mice were divided into NNS-administered, sucrose-administered (33% sucrose in drinking water), and control-administered (normal water) groups. The commercially available NNS (Sigma, Tokyo, Japan) contained 99% erythritol and 1% aspartame, and was added to the drinking water at a dosage of 4% for 4 weeks. The rationale for choosing the doses of sucrose and NNS was based on previous studies [26–28] and our preliminary study. For each of the 4 weeks of treatment, food intake and water consumption were measured every 24 h. In addition, body weight was measured at 16:00–17:00 h each day. After treatment for 4 weeks, WAT and interscapular BAT were removed, frozen in liquid nitrogen, and stored at −80 °C. Epididymal WAT and BAT, liver histology, and the levels of metabolic factors were assessed in all animals.

2.3. Blood sampling and analysis

Blood was collected after a 6-h fast. Serum was separated and frozen immediately at −20 °C until assayed. Serum levels of glucose, insulin, and triglycerides (TGs) were measured using commercially available kits (Wako Chemical, Tokyo, Japan). To test glucose tolerance, each mouse was intraperitoneally injected with glucose at a dose of 1.0 mg/g body weight after a 6-h fast.

2.4. Histological analysis

Small pieces of liver and epididymal WAT and BAT were dissected, washed in saline, fixed in 10% formalin, and embedded in paraffin. Tissue sections were cut at a thickness of 5 μm and stained with hematoxylin and eosin.

2.5. Tissue triglyceride levels

One hundred milligrams of skeletal muscle and liver was homogenized respectively in 2 mL of a solution containing 150 mmol/L NaCl, 0.1% Triton X-100, and 10 mmol/L Tris, using a Polytron homogenizer (NS-310E; Micro Tech Nichion, Chiba, Japan) for 1 min. The TG content was determined using a commercially available kit (Wako Chemical).

2.6. Western blot analysis

Frozen tissue samples were homogenized in sodium dodecyl sulfate sample buffer, centrifuged, and boiled. The total protein concentrations of the tissue samples were quantified by the Bradford method. Equal amounts of total protein were loaded onto 8% sodium dodecyl sulfate-polyacrylamide gels, electrophoresed, and then transferred electrophoretically to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Richmond, CA). The membranes were blocked with 5% nonfat milk for 1 h; incubated overnight with primary antibodies against UCPs, peroxisome proliferator-activated receptors (PPARs), peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1), carnitine palmitoyltransferase 1 (CPT-1),

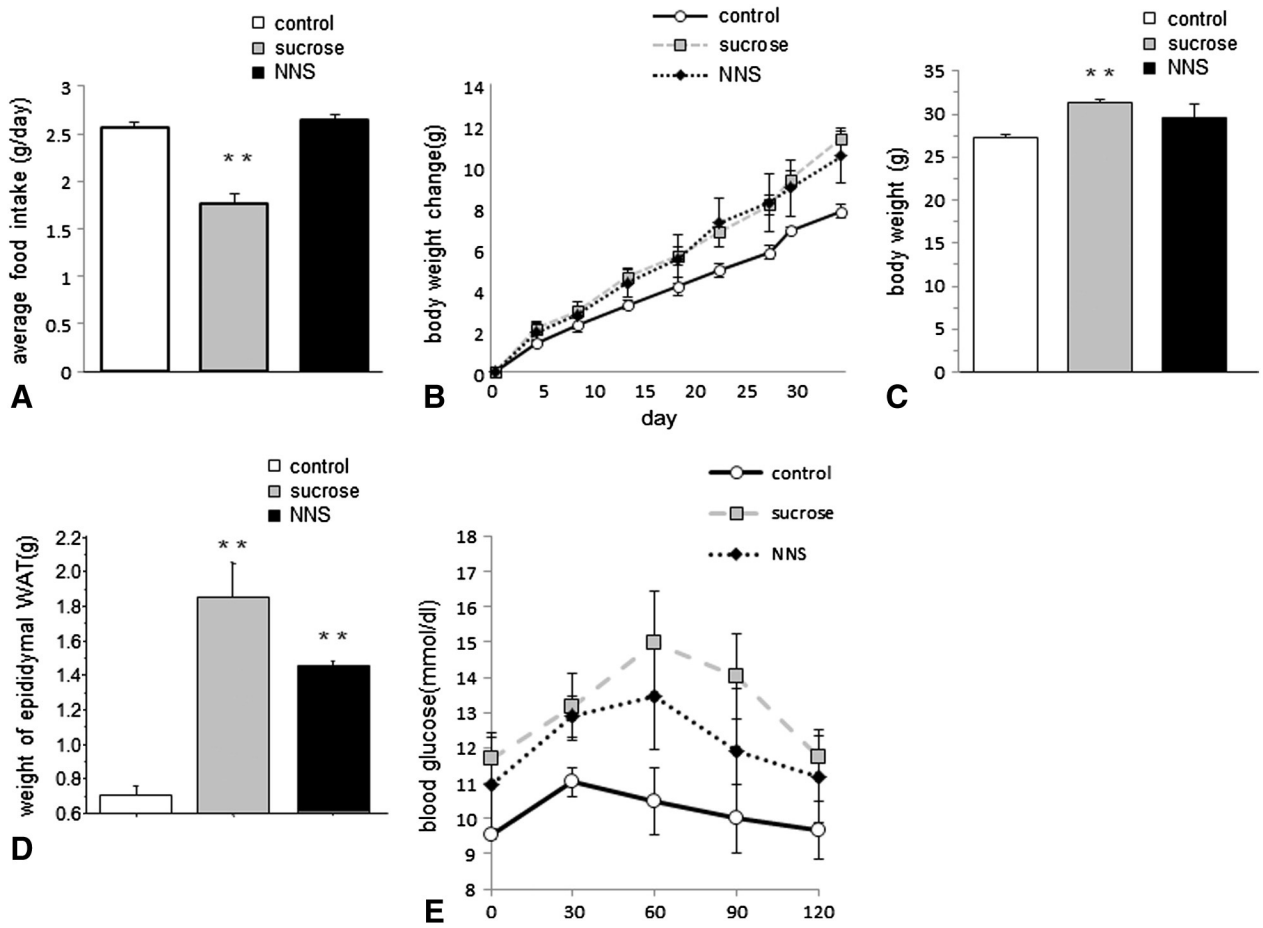

Fig. 1 – Effects of sucrose and NNS supplementation on average food intake (A), body weight change (B), final body weight (C), weight of epididymal WAT (D), and the result of the intraperitoneal glucose tolerance test (E) in mice with diet-induced obesity. Data are means ± SEM. * $P < 0.05$, $P < 0.01$ vs. control; n = 5. Differences in multiple comparisons were analyzed using an ANOVA post-hoc test.**

leptin, fatty acid synthetase (FAS), proopiomelanocortin (POMC), neuropeptide Y (NPY), and tubulin (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C; and then incubated for 1 h at room temperature with the secondary antibody. Proteins were detected by enhanced chemiluminescence (Amersham Life Science, Buckinghamshire, UK) and quantitated using imaging software (Bio-Rad Laboratories).

2.7. *Locomotor activity*

NNS or sucrose was administered, and then locomotor activity was examined. Samples were collected approximately every 30 min for 3 days after adaptation. For each time point, the values for the samples in each group were averaged.

2.8. *Indirect calorimetry*

In vivo indirect calorimetry was performed using an Oxymax system (no. 05142; Columbus Instruments, Columbus, OH). A constant airflow (0.6 L/min) was applied to the chambers and monitored by a flow meter. To calculate the oxygen consumption (VO_2), carbon dioxide production, and respiratory quotient (RQ; ratio of carbon dioxide production to VO_2), gas concentrations were monitored at the inlet and outlet of each sealed chamber. Treated animals were randomly placed into the experimental chamber at 25 ± 1 °C and 55% ± 5% humidity, with free access to food and water. The mice were individually housed in Plexiglas cages, through which air of known O_2 concentration was passed at a constant flow rate. After a 24-h acclimation period, exhaust air was sampled from mice in the fed state to determine O_2 and CO_2 levels. After confirming the stability of VO_2 and RQ, the NNS or sucrose was administered, and then calorimetry was performed. Five days after administration, samples were collected approximately every 10 min. For each time point, the values for the samples in each group were averaged.

2.9. *Statistical analyses*

All data are expressed as means ± SEM. Differences in multiple comparisons were analyzed using an ANOVA post hoc test (StatView 5.0; SAS Institute, Cary, NC).

3. Results

3.1. *Effects of sucrose and NNS supplementation on food intake and body weight in mice with diet-induced obesity*

The average food intake, average change in body weight, and final body weight are shown in Fig. 1A–C for mice with diet-induced obesity. Compared to the controls, sucrose supplementation decreased daily food intake but increased body weight in obese mice. In contrast, there was no significant difference in average food intake ($P > 0.1$) or body weight gain ($P > 0.1$) between NNS-administered and control obese mice ($P > 0.1$).

3.2. *Effects of sucrose and NNS supplementation on WAT weight, WAT morphology, and WAT leptin*

Compared with the control supplementation, sucrose supplementation increased WAT weight (Fig. 1D) and WAT cell size (Fig. 2A) in mice. NNS supplementation increased WAT weight compared with controls ($P < 0.01$). The TG content was increased in the liver and skeletal muscle of NNS- and sucrose-administered mice compared to control mice ($P < 0.05$ for each) (Table 1). The WAT leptin level was significantly increased in NNS-treated obese mice compared to control mice ($P < 0.05$) (Fig. 2B).

3.3. *Effects of sucrose and NNS supplementation on serum glucose, insulin, and TG levels*

Compared with the control supplementation, sucrose supplementation significantly increased the serum levels of glucose and insulin ($P < 0.05$ or $P < 0.01$) (Table 1). The serum glucose level in the NNS group was lower than that in the sucrose group ($P < 0.01$) (Table 1). Interestingly, the serum insulin level was increased in the NNS group compared with the control group ($P < 0.05$) (Table 1). The serum TG level was higher in sucrose-administered obese mice than in the control and NNS groups ($P < 0.05$ for each) (Table 1).

3.4. *Effects of sucrose and NNS supplementation on the glucose tolerance test*

Compared to controls, sucrose supplementation significantly increased the serum levels of glucose (Table 1). The levels in the NNS group were higher than those in controls 60 min after glucose loading ($P < 0.05$) (Fig. 1E). The area under the blood concentration–time curve was increased in both the sucrose group and NNS group.

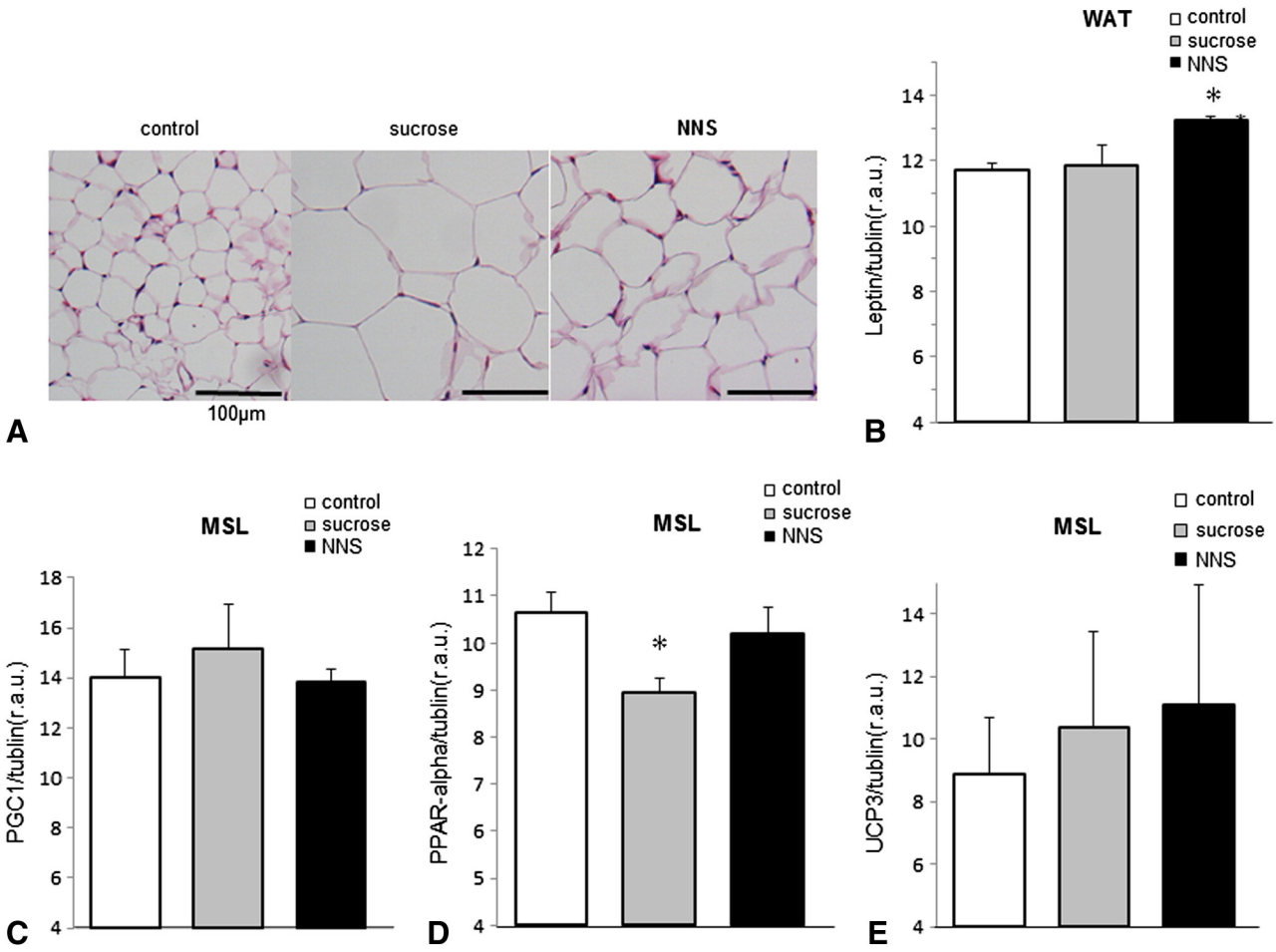

Fig. 2 – Effects of sucrose and NNS supplementation on histology of epididymal WAT (A), WAT leptin level (B), MSL PGC1 level (C), MSL PPAR-alpha level (D), and MSL UCP3 level (E) in mice with diet-induced obesity. Data are means ± SEM. r.a.u., relative arbitrary units. * $P < 0.05$ vs. control; n = 5. Differences in multiple comparisons were analyzed using an ANOVA post-hoc test.

Table 1 – The levels of serum glucose, serum insulin, serum and tissue triglyceride (TG) by sucrose and NNS treatment.

	Glucose (mg/dL)	Insulin (ng/mL)	TG (mg/dL)	liver TG (mg/dL)	muscle TG (mg/dL)
Control	152 ± 9	0.7 ± 0.1	37.0 ± 4.6	19.5 ± 1.4	2.3 ± 0.4
Sucrose	282 ± 17**	1.7 ± 0.2**	59.0 ± 5.2*	37.5 ± 3.8*	5.5 ± 0.5*
NNS	177 ± 6##	1.5 ± 0.3*	49.5 ± 4.3	34.5 ± 4.9*	4.8 ± 0.4*

Significant difference at *$p < 0.05$, **$p < 0.01$ versus control; ##$p < 0.01$ versus sucrose.
NNS: nonnutritive sweetener, TG: triglyceride.

3.5. *Effects of NNS supplementation on lipid metabolism and energy metabolism in the liver*

Compared to controls, sucrose and NNS supplementation increased fat deposition and TG levels in the liver (Fig. 3E, Table 1). The FAS level was increased by sucrose supplementation ($P < 0.05$) (Fig. 3A). However, there was no significant difference in liver UCP2, PPAR-alpha, CPT-1, or FAS levels between the NNS and control groups ($P > 0.1$ for each) (Fig. 3A–D).

3.6. *Effects of NNS supplementation on lipid metabolism and energy metabolism in skeletal muscle*

Compared to the controls, sucrose and NNS supplementation increased TG levels in skeletal muscle (Table 1). The levels of PGC-1 and UCP3 in skeletal muscle were not significantly changed by NNS supplementation ($P > 0.1$ for each) (Fig. 2C, E). Compared to the controls, sucrose supplementation significantly decreased the level of PPAR-alpha ($P < 0.05$) (Fig. 2D).

3.7. *Effects of NNS supplementation on proopiomelanocortin (POMC) and neuropeptide Y (NPY)*

Supplementation of NNS did not change the levels of POMC or NPY in the brain among the groups ($P > 0.1$).

3.8. *Effects of NNS supplementation on BAT UCP1, PPAR-alpha, and PGC-1 levels and BAT morphology*

In obese mice, the BAT UCP1 level was lower in the sucrose and NNS groups than in the control group ($P < 0.05$ or $P < 0.01$)

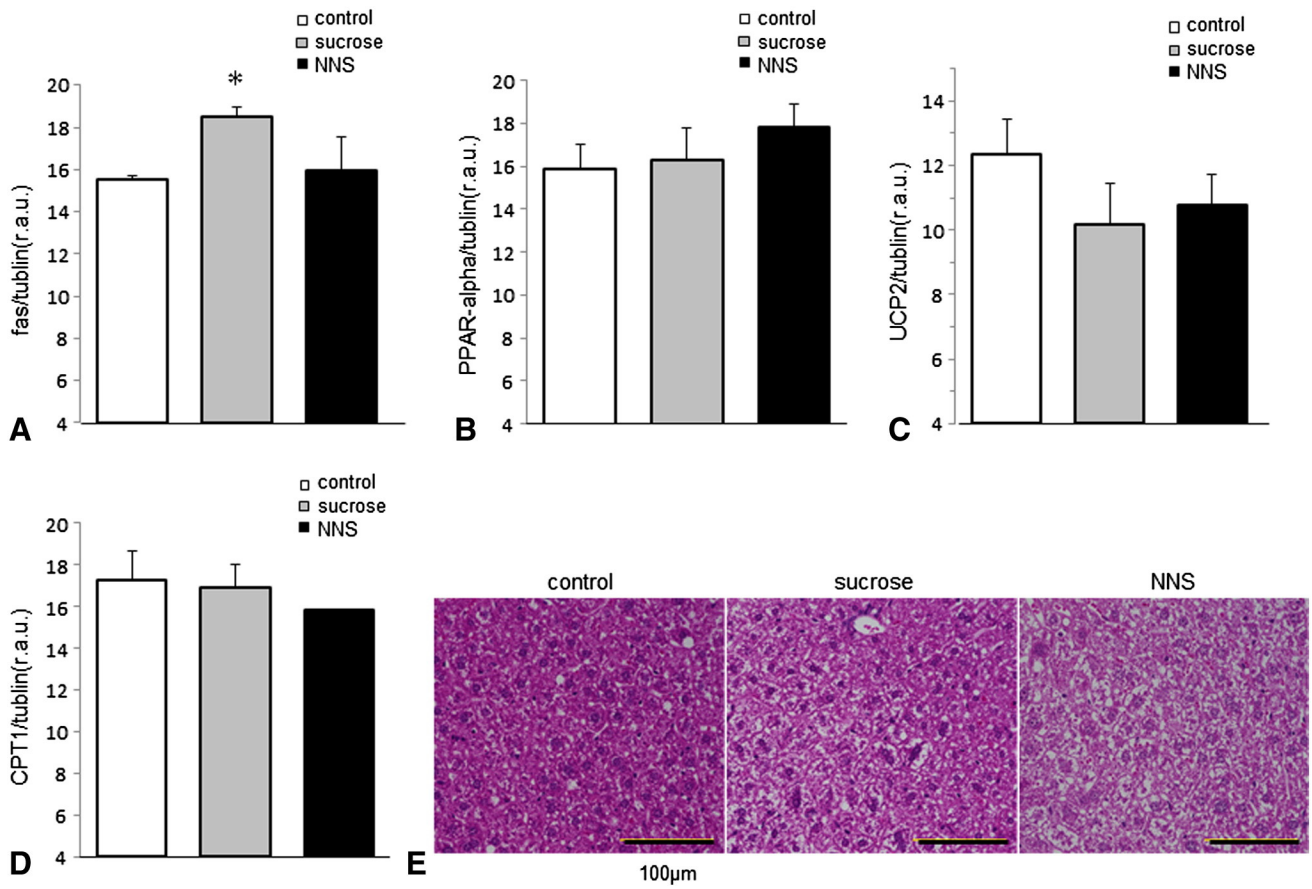

Fig. 3 – Effects of sucrose and NNS supplementation on the FAS level (A), PPAR-alpha level (B), UCP2 level (C), CPT1 level (D), and histology (D) of liver in mice with diet-induced obesity. Data are means ± SEM. r.a.u., relative arbitrary units. * $P < 0.05$ vs. control; n = 5. Differences in multiple comparisons were analyzed using an ANOVA post-hoc test.

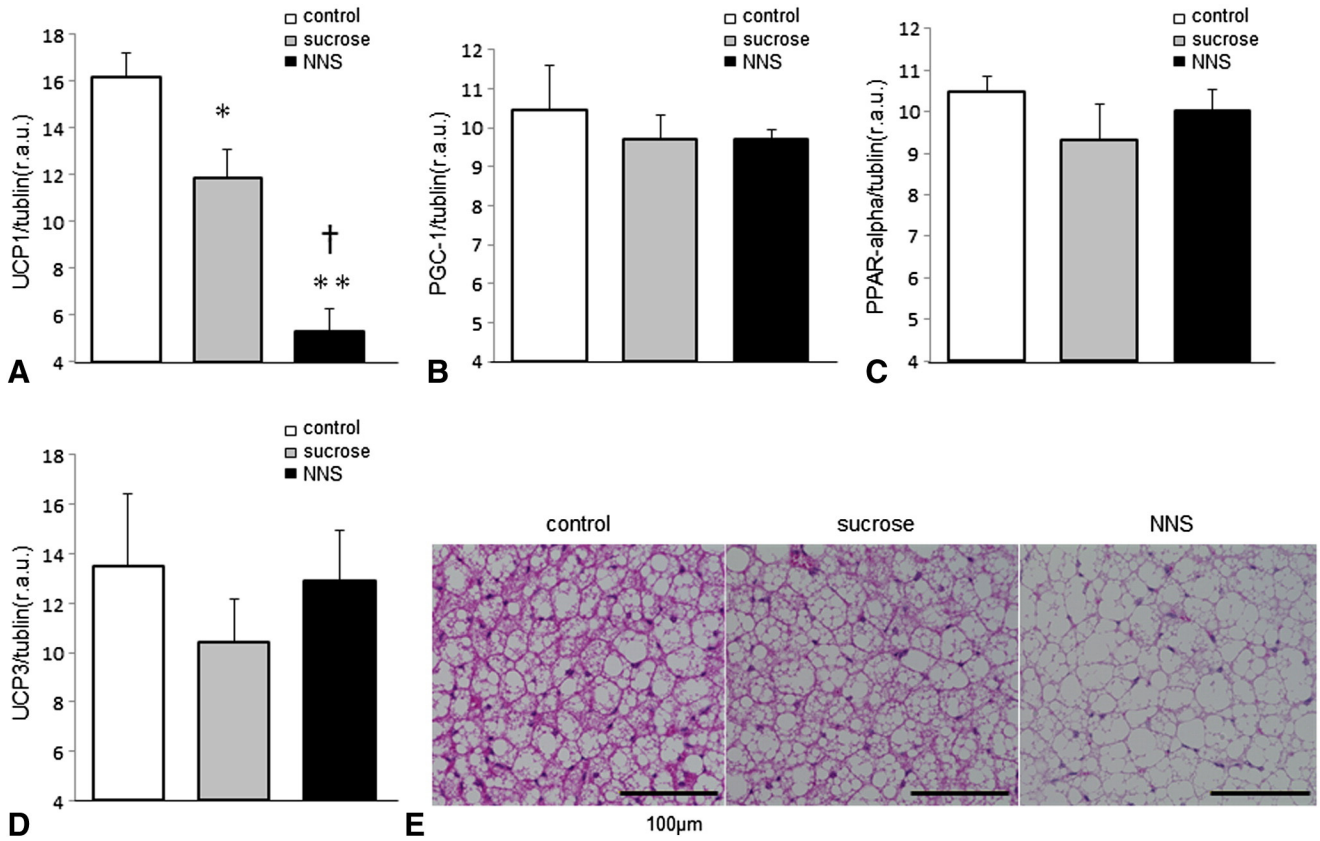

Fig. 4 – Effects of sucrose and NNS supplementation on the UCP1 level (A), PGC-1 level (B), PPAR-alpha level (C), UCP3 level (D), and histology (E) of BAT in mice with diet-induced obesity. Data are means ± SEM. r.a.u., relative arbitrary units. * $P < 0.05$, ** $P < 0.01$ vs. control; † $P < 0.05$ vs. sucrose; n = 5. Differences in multiple comparisons were analyzed using an ANOVA post-hoc test.

(Fig. 4A). In addition, the BAT UCP1 level was lower in the NNS group than in the sucrose group ($P < 0.05$) (Fig. 4A). There was no significant difference in the BAT PPAR-alpha, PGC-1 or UCP3 levels between the NNS and control groups ($P > 0.1$) (Fig. 4B–D). Compared with the controls, the sucrose and NNS supplementation increased fat deposition in BAT (Fig. 4E).

3.9. *Effects of NNS supplementation on locomotor activity*

The high-sucrose group showed more locomotor activity under both light and dark conditions than the other groups ($P < 0.01$ for each comparison) (Fig. 5A–F). NNS supplementation increased locomotor activity compared to controls, but only during the light phase ($P < 0.01$) (Fig. 5F).

3.10. *Effects of NNS supplementation on oxygen consumption and RQ*

Compared to controls, sucrose supplementation increased oxygen consumption under both light and dark conditions ($P < 0.01$ for each) (Fig. 6A–F). Conversely, NNS supplementation decreased oxygen consumption compared to the other groups during the light phase (Fig. 6A–C). NNS supplementation decreased the RQ compared to the sucrose group during both the light and dark phases (Fig. 6D–F).

4. Discussion

The present study examined the effects of sucrose and NNS supplementation on food intake, body weight, and metabolic parameters in mice with DIO. As expected, sucrose supplementation significantly increased body adiposity and glucose and insulin levels. Daily food intake was decreased by sucrose supplementation. This suggests that a high-glucose and high-calorie diet influences appetite and consequently decreases food consumption.

Compared with sucrose supplementation, NNS supplementation decreased the serum glucose level. Interestingly, compared with the control treatment, NNS supplementation increased the serum insulin level in mice with DIO. In addition, NNS administration influenced glucose tolerance compared to controls. These observations suggest that NNS supplementation induced insulin resistance by increase of tissue triglyceride, although some NNSs are used to control hyperglycemia.

NNS supplementation increased the WAT leptin level in DIO mice in the present study. It is possible that the high leptin level was related to body adiposity. Indeed, NNS administration increased the weight of epididymal fat. Thus, it is possible that the high leptin level was related to the influence on body adiposity.

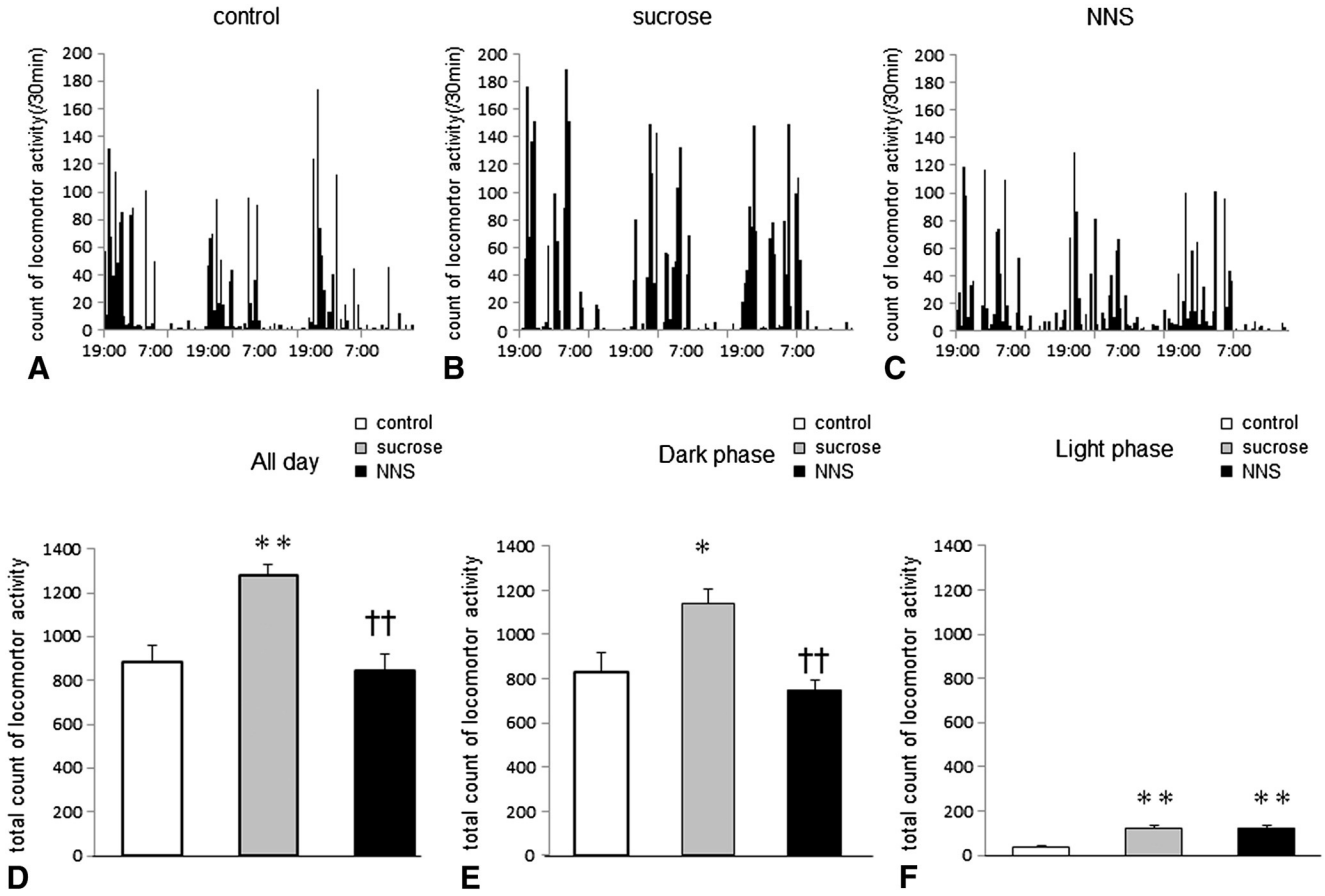

Fig. 5 – Effects of sucrose and NNS supplementation on locomotor activity per 30 min for 3 days (A-C) and total locomotor activity all day (D), in the dark (E), and in the light (F) in mice with diet-induced obesity. Data are means ± SEM. * P < 0.05, ** P < 0.01 vs. control; †† P < 0.01 vs. sucrose; n = 5. Differences in multiple comparisons were analyzed using an ANOVA post-hoc test.

In previous studies, NNS administration increased food intake [29,30]. However, in the present study, NNS supplementation increased body adiposity without affecting food intake. NNS supplementation at the dose used in the present study might have been insufficient to induce hyperphagia. Alternatively, differences in NNS types and/or experimental schedules among the studies may be responsible for the different results.

Considering that NNS supplementation did not affect food intake in the present study, the increase in body adiposity in mice with DIO may be attributable to an effect on energy metabolism or lipolysis. Compared to the controls, NNS supplementation decreased oxygen consumption, but not daily locomotor activity. A decrease in oxygen consumption without a concomitant decrease in carbohydrate intake may reflect reduced sympathetic activity. These observations suggest that a change in oxygen consumption caused by NNS supplementation may regulate body adiposity by affecting energy metabolism.

A number of factors that affect energy metabolism have been described, including UCPs [17,18,22]. UCPs are mitochondrial transporters located in the inner mitochondrial membrane [22,31]. The first member of the family, UCP1, is expressed in brown adipocytes and is responsible for the thermogenic capacity of BAT. UCP1 enhances proton conductivity through the mitochondrial inner membrane, thereby uncoupling the respiratory chain and heat production. This action of UCP1 in BAT constitutes the main molecular basis for non-shivering thermogenesis in rodents in response to cold exposure and diet [17,31]. The thermogenic activity of brown fat is regulated primarily by norepinephrine, which is released from the sympathetic nervous system innervating the tissue and acts through beta-adrenergic, cAMP-dependent pathways [17].

Evidence that has accumulated during the past two decades indicates that energy expenditure processes elicited by UCP1 are involved in the control of energy balance and that UCP1 activity in BAT may provide the basis for diet-induced thermogenesis [32]. In the present study, NNS supplementation decreased the level of UCP1 in BAT compared with the level in controls. Conversely, NNS did not significantly influence the energy homeostatic marker in the liver, muscle, and brain. This indicates that NNS supplementation may partly regulate energy metabolism through BAT UCP1.

Here, we must consider the question of why NNS decreases UCP1. As mentioned above, NNS supplementation increased the WAT leptin level, indicating the existence of leptin resistance in mice. In general, treatment of leptin induced

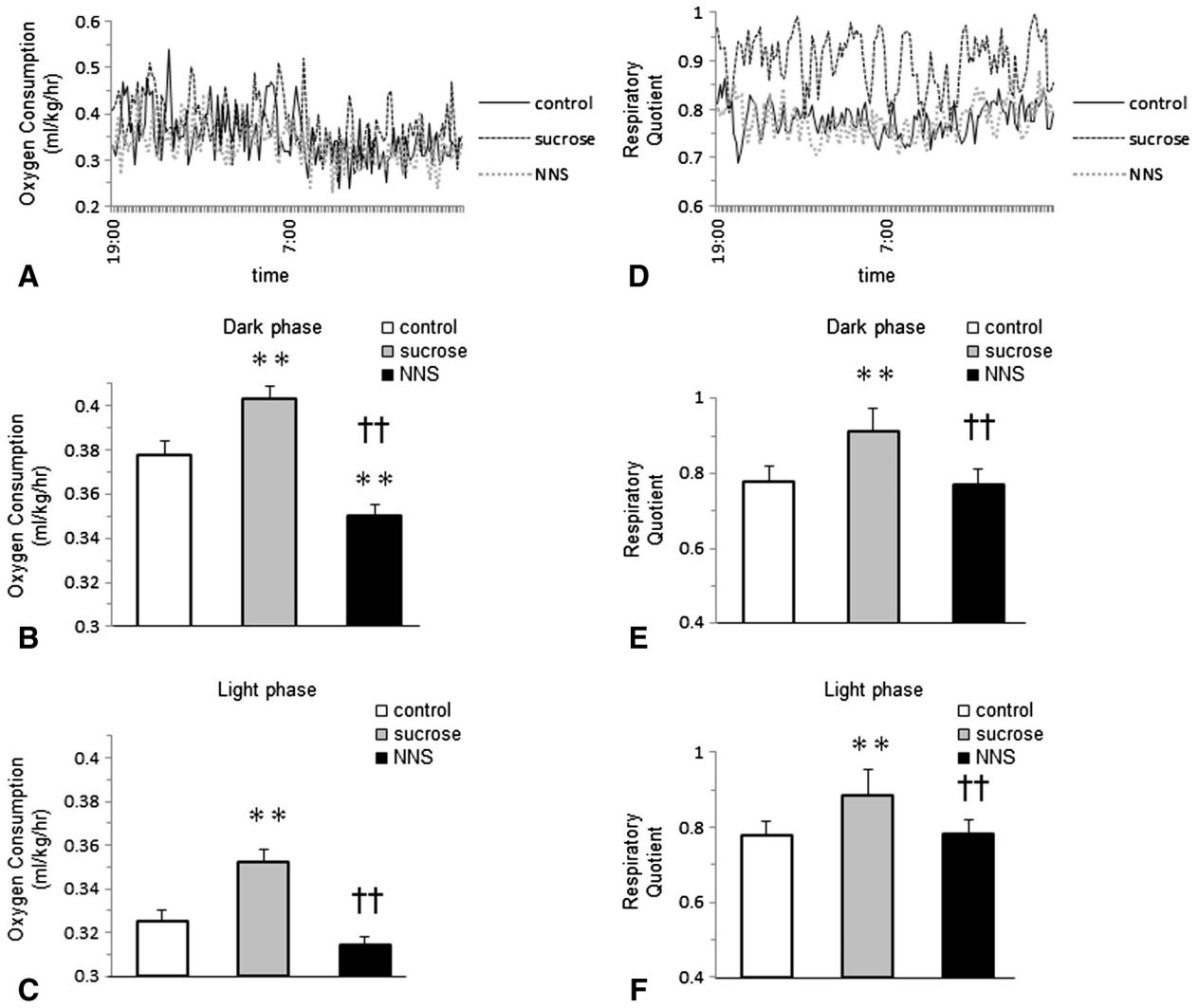

Fig. 6 – Effects of sucrose and NNS treatments on oxygen consumption in all phases (A), dark phase (B), and light phase (C), and on RQ in all phases (D), dark phase (E), and light phase (F) in mice with diet-induced obesity. Data are means ± SEM. ** P < 0.01 vs. control; †† P < 0.01 vs. sucrose; n = 5. Differences in multiple comparisons were analyzed using an ANOVA post-hoc test.

BAT UCP1, suggesting that leptin resistance decreased the levels of BAT UCP1 [33]. Thus, leptin resistance by NNS might have influenced BAT UCP1 in the present study.

It is interesting that the NNS supplementation affected body adiposity and several other metabolic factors only in obese mice. It is possible that the combination of an NNS and a high-fat diet can influence energy metabolism. In addition, several fatty acids and/or other factors may alter the effects of an NNS in obese mice. Further studies are needed to clarify this point.

This study has several limitations. First, the effects of NNSs are difficult to identify. Most studies have indicated that NNSs help to control body weight and improve related metabolic disorders [3–5]. However, other studies [9,34], including the present one, suggest that NNSs may paradoxically lead to weight gain. Findings regarding the influence of NNSs have been inconsistent, possibly due to differences in NNS types, experimental designs, and clinical protocols, including types, doses, delivery, combinations, vehicles, and duration of administration. Well-designed and controlled studies are needed to determine the long-term effects of NNSs on body weight regulation and glycemic control at different points in the life cycle. Second, the detailed mechanisms by which NNSs influence obesity, leptin resistance, and BAT UCP1 must be examined. Third, the interference of NNS in glucose and lipid metabolism with mimetic action must be examined.

In summary, the tested NNS increased body adiposity and decreased the BAT level of UCP1 in DIO mice.

Author contributions

Kimihiko Mitsutomi and Takayuki Masaki were responsible for designing and conducting the study, interpreting the data, and writing the manuscript. Takanobu Shimasaki contributed to data collection, analysis, and interpretation. Koro Gotoh, Seiichi Chiba, Tetsuya Kakuma and Hirotaka Shibata contributed to data interpretation.

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