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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),