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Metabolic effects of intermittent access to caloric or non-caloric sweetened solutions in mice fed a high-caloric diet

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ABSTRACT

Human consumption of obesogenic diets and soft drinks, sweetened with different molecules, is increasing worldwide, and increases the risk of metabolic diseases. We hypothesized that the chronic consumption of caloric (sucrose, high-fructose corn syrup (HFCS), maltodextrin) and non-caloric (sucralose) solutions under 2-hour intermittent access, alongside the consumption of a high-fat high-sucrose diet, would result in differential obesity-associated metabolic abnormalities in mice.

Male C57BL/6 mice had ad libitum access to an HFHS diet and to water (water control group). In addition, some mice had access, 2 h/day, 5 days/week (randomly chosen) for 12 weeks, to different solutions: i) a sucrose solution (2.1 kJ/ml), ii) an HFCS solution (2.1 kJ/ml), iii) a maltodextrin solution (2.1 kJ/ml) and a sucralose solution (60 mM) (n = 15/group).

Despite no changes in total caloric intake, 2 h-intermittent access to the sucrose, HFCS or maltodextrin solutions led to increased body weight and accumulation of lipids in the liver when compared to the group consuming water only. The HFCS and sucrose solutions induced a higher fat mass in various fat depots, glucose intolerance, increased glucose oxidation at the expense of lipid oxidation, and a lower hypothalamic expression of NPY in the fasted state. HFCS also reduced proopiomelanocortin expression in the hypothalamus. 2 h-intermittent access to sucralose did not result in significant changes in body composition, but caused a stronger expression of CART in the hypothalamus. Finally, sucrose intake showed a trend to increase the expression of various receptors in the nucleus accumbens, linked to dopamine, opioid and endocannabinoid signaling.

In conclusion, 2 h-intermittent access to caloric solutions (especially those sweetened with sucrose and HFCS), but not sucralose, resulted in adverse metabolic consequences in high-fat high-sucrose-fed mice.

1. Introduction

Human consumption of obesogenic diets is associated with an intake of sugar-sweetened beverages [31,34], that is increasing worldwide. Among these beverages, those sweetened with sucrose and high-fructose corn syrup (HFCS) increase both caloric intake and body weight in humans [7,11,28,38,44]. In rodents fed control (low-fat) diets, beverages sweetened with sucrose [9,10,18,19,40], fructose [6,16,26,27,29] or maltodextrin (a mildly-sweetened carbohydrate that does not contain fructose) [20] also usually induce body weight gain, although this has not been observed in some studies with sucrose [6,16,23,24] or fructose [33]. Interestingly, and in contrast to fructose supplied in a liquid form, long-term high-fructose feeding through solid foods does not lead to increased weight gain in mice [45]. In view of this important impact of traditional caloric sweeteners on body weight, non-caloric and high-intensity sweeteners such as sucralose and saccharin [39,48] are proposed as an alternative to reduce the caloric content of foods and beverages [14,32,43]. However, several epidemiological studies have found that the consumption of these non-caloric sweeteners is associated with increased rates of obesity and diabetes [35–37,42,48] and their impact on metabolism requires further research [17,30]. In regard to these conflicting data, our aim was to study

Abbreviations: HFCS, High-Fructose Corn Syrup; HDL, High-density lipoprotein; TG, triglycerides; FFA, Free Fatty Acids; PYY, Peptide YY; NAcc, Nucleus Accumbens; POMC, Proopiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; AgRP, Agouti-related peptide; NPY, Neuropeptide Y; MC4R, Melanocortin receptor 4; DA, Dopamine; DR, Dopamine Receptor; OR, Opioid-receptor; GIP, Gastric inhibitory polypeptide; GLP-1, Glucagon-like Peptide-1; CB1-R, Endocannabinoid Receptor 1; DIO, Diet-induced obesity

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the effect of solutions sweetened with different caloric and non-caloric sweeteners in one experimental design.

In terms of peptide secretion, sucrose solutions are known to decrease the expression of PYY in the serum after 2 weeks of consumption [27], and to affect the expression of different neuropeptides and their receptor systems in the hypothalamus [2,24,40] and reward system [1,4]. The long term effects of other caloric (HFCS, maltodextrin) and non-caloric (sucralose, saccharin) sweeteners on gut peptide secretion [8] remain unclear.

Previous experiments have shown that 2 h-intermittent access to a sucrose solution could aggravate the effects of a high-fat diet on energy homeostasis [40]. The present study was designed to determine whether the intermittent consumption of different sweeteners might affect body metabolism and peripheral and central pathways in mice fed a high-fat high-sucrose western diet. This study focused on the sweeteners found in human beverages that are either caloric (sucrose, HFCS, maltodextrin) or non-caloric (sucralose). Maltodextrin served as a control for caloric content (as it is not as sweet as sucrose) and sucralose as a control for sweetness. Measurements included body weight and body composition, feeding and drinking patterns, rates of glucose and lipid oxidation measured by indirect calorimetry, glucose tolerance, circulating lipid metabolites, and the expression of gut peptides and molecules involved in the central homeostatic and food reward pathways.

2. Methods

2.1. Animals and diet

75 male C57Bl/6J mice (Harlan Laboratories, France), aged 5 weeks at their arrival in the laboratory, were housed individually in standard laboratory cages (with a wired mesh floor), in a temperature-controlled room (22 ± 1 °C) maintained on a reversed 12:12 h-dark cycle (lights off at 09:30) for the whole study. The animals were given ad libitum access to standard chow and water for one week to adapt to the laboratory conditions. For the next 3 weeks, they were fed a high-fat high-sucrose diet (AIN93M modified diet, UPAE, INRA Jouy-en-Josas) that provided 19 kJ/g of energy (14% protein, 41% carbohydrate (50% starch, 50% sucrose) and 45% fat (95% lard), Table S1) [40]. This diet was moistened (70/30 ratio of powder/water) to minimize spillage. During the experiment, food and water were available ad libitum for 7.5 h/day (09:30–17:00 – the first 8.5 h of the night period), as used during previous experiments [40]. We used this time-limited access to mimic human feeding patterns, as people mostly eat during the 'active' period. Food was available immediately at the beginning of the dark cycle to avoid any potential metabolic stress due to restricting food intake when the lights were turned off. This food-limitation paradigm didn't induce any change in body weight compared to unlimited access in chow-fed mice (unpublished data). The study was approved by the French National Animal Care Committee (Approval number 12/088) and conformed to the European legislation on the use of laboratory animals.

2.2. Design

The mice were divided randomly into five weight-matched groups (n = 15/group). Control mice had access to the high-fat high-sucrose diet and water only for 12 weeks. The four other groups had access to the high-fat high-sucrose diet and water, plus 2 h-intermittent random access to a solution containing either (i) 12.6% sucrose (w/v dissolved in tap water, 2.1 kJ/ml), (ii) 16.7% high-fructose corn syrup (HFCS) (Nature's Flavors®, Formula 55, w/v dissolved in tap water, 2.1 kJ/ml), (iii) 13% maltodextrin (Caloreen®, Nestlé, w/v dissolved in tap water, 2.1 kJ/ml) or (iv) 60 mM sucralose (Splenda®, no calories). These solutions were available for 2 h/day (a randomly chosen 2 h-period during the 7.5 h-period when food and water were available) and only 5 days/week (the days were also randomly chosen), so that access was intermittent and unpredictable.

2.3. Body weight (BW) and body composition

The mice were weighed twice a week between 08:30 and 09:30 (before the lights went off). Body fat and lean mass were measured every 2 weeks by dual energy X-ray absorptiometry using a Piximus apparatus (Lunar Piximus, GE Medical Systems).

2.4. Measurement of the feeding pattern, spontaneous activity and rates of glucose and lipid oxidation

The intakes of food and sweetened solutions were measured twice a week by determining changes in the weight of individual cups containing the food or solutions (placed on a grid floor). The data were corrected for spillage, food moistening and evaporation. The two weekly measurements of food and drink intake were averaged and converted into kJ before statistical analysis. Detailed recordings of food (FI) and drink intake patterns, spontaneous physical activity (SPA) and VO_2 and VCO_2 levels were obtained using individual metabolic cages during weeks 8 and 9, for 2 or 3 consecutive days, during which access to the high-fat high-sucrose diet was available from 09:00 to 17:00, as usual. Day 1 in the metabolic cage was used for habituation. Recordings were taken during day 2 for all groups, and for standardization, sweetened solutions were made available between 11:00 and 13:00. Recordings were only taken on day 3 for mice with intermittent access to sweetened solutions, in order to study their feeding and SPA patterns in the absence of access to the solutions. VO_2 and VCO_2 were measured on each cage for 100 s every 10 min. Food intake, drink intake and SPA (activity that includes all movement) data, initially recorded at 2 s intervals, were pooled into 10-min intervals. Food/drink intake data were then analysed to extract the following parameters: numbers of bouts, size of bouts (kJ), bout duration (min), ingestion speed (kJ/min) and inter-meal interval (min) (as described previously (40)). Glucose oxidation (Gox) and lipid oxidation (Lox) profiles were computed in Watts (J/s) from the VO_2 and VCO_2 values (ml/min).

2.5. Oral glucose tolerance test (OGTT)

During week 11, an oral glucose load (2 g/kg) was delivered to each mouse via oral gavage after an overnight fast. Blood samples were taken from the tail before administration of the glucose load and 15, 30, 60 and 120 min after the load, and blood glucose was measured using a glucometer (LifeScan, One-Touch Vita). The rest of the blood collected was centrifuged immediately (3000 g, 4 °C, 15 min) and stored at − 80 °C to determine insulin levels (Mercodia Mouse Insulin ELISA).

2.6. Plasma/tissue collection and analysis

At week 13, mice were fasted overnight. 90 min before anaesthesia (2.5% isoflurane in 1.2 L/min O_2), half of the mice in each group remained fasted while the other half received 0.6 ml (1.3 kJ) of their specific solutions. Mice were then killed by decapitation. Fat (subcutaneous, retroperitoneal, inguinal, and mesenteric), lean tissues and organs (liver, spleen, kidneys, lungs, brain, heart) were dissected out, blotted dry, and weighed to the nearest 0.01 g. The duodenum, jejunum and ileum were flushed with iced-cold sterile PBS then gently scraped off and frozen in Trizol in liquid nitrogen. The brain was quickly removed and the hypothalamus dissected and snap-frozen in Trizol® reagent. The nucleus accumbens (NAcc) was harvested using a cooled mouse brain matrix (Braintree Scientific, INC) then snap-frozen in Trizol® and stored at − 80 °C. Blood was collected in EDTA collection tubes containing a cocktail of anti-proteases (Complete mini, Roche, Germany), centrifuged (3000 g, 4 °C, 15 min) and the plasma was stored at − 80 °C. Plasma levels of cholesterol, high-density lipoprotein