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Liquid Sucrose Consumption Promotes Obesity and Impairs Glucose Tolerance Without Altering Circulating Insulin Levels

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For chronic weight management in adults with a BMI of ≥30 kg/m² (obesity), or ≥27 kg/m² (overweight) in the presence of a weight-related comorbidity, as an adjunct to a reduced calorie diet and increased physical activity.

Indications and Usage
Wegovy™ (semaglutide) injection 2.4 mg is indicated as an adjunct to a reduced calorie diet and increased physical activity for chronic weight management in adults with an initial body mass index (BMI) of ≥30 kg/m² (obesity) or ≥27 kg/m² (overweight) in the presence of at least one weight-related comorbid condition (e.g., hypertension, type 2 diabetes mellitus, or dyslipidemia).

Limitations of Use
- Wegovy™ contains semaglutide and should not be coadministered with other semaglutide-containing products or with any GLP-1 receptor agonist.
- The safety and effectiveness of Wegovy™ in combination with other products intended for weight loss, including prescription drugs, over-the-counter drugs, and herbal preparations, have not been established.
- Wegovy™ has not been studied in patients with a history of pancreatitis.

Important Safety Information

WARNING: RISK OF THYROID C-CELL TUMORS
- In rodents, semaglutide causes dose-dependent and treatment-duration-dependent thyroid C-cell tumors at clinically relevant exposures. It is unknown whether Wegovy™ causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans as human relevance of semaglutide-induced rodent thyroid C-cell tumors has not been determined.
- Wegovy™ is contraindicated in patients with a personal or family history of MTC or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2). Counsel patients regarding the potential risk for MTC with the use of Wegovy™ and inform them of symptoms of thyroid tumors (e.g., a mass in the neck, dysphagia, dyspnea, persistent hoarseness). Routine monitoring of serum calcitonin or using thyroid ultrasound is of uncertain value for early detection of MTC in patients treated with Wegovy™.

Contraindications
- Wegovy™ is contraindicated in patients with a personal or family history of MTC or in patients with MEN 2, and in patients with a prior serious hypersensitivity reaction to semaglutide or to any of the excipients in Wegovy™. Serious hypersensitivity reactions, including anaphylaxis and angioedema have been reported with semaglutide.

Warnings and Precautions
- Risk of Thyroid C-Cell Tumors: Patients should be further evaluated if serum calcitonin is measured and found to be elevated or thyroid nodules are noted on physical examination or neck imaging.
- Acute Pancreatitis: Acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis, has been observed in patients treated with GLP-1 receptor agonists, including semaglutide. Acute pancreatitis was observed in patients treated with Wegovy™ in clinical trials. Observe patients carefully for signs and symptoms of acute pancreatitis (including persistent severe abdominal pain, sometimes radiating to the back, and which may or may not be accompanied by vomiting). If acute pancreatitis is suspected, discontinue Wegovy™ promptly, and if acute pancreatitis is confirmed, do not restart.
- Acute Gallbladder Disease: In clinical trials, cholecystitis was reported by 1.6% of Wegovy™ patients and 0.7% of placebo patients. Cholecystitis was reported by 0.6% of Wegovy™ patients and 0.2% of placebo patients. If cholecystitis is suspected, discontinue, gallbladder studies and appropriate clinical follow-up are indicated.
- Hypoglycemia: Wegovy™ lowers blood glucose and can cause hypoglycemia. In a trial of patients with type 2 diabetes, hypoglycemia was reported in 6.2% of Wegovy™ patients versus 2.5% of placebo patients. Patients with type 2 diabetes taking Wegovy™ with an insulin secretagogue (e.g., sulfonylurea) or insulin may have an increased risk of hypoglycemia, including severe hypoglycemia. Inform patients of the risk of hypoglycemia and educate them on the signs and symptoms. Monitor blood glucose in patients with type 2 diabetes.
- Acute Kidney Injury: There have been postmarketing reports of acute kidney injury and death in patients with chronic renal failure, which in some cases required hemodialysis, in patients treated with semaglutide. Patients with renal impairment may be at a greater risk of acute kidney injury, but some events have been reported in patients without known chronic kidney disease or renal failure. A majority of the events occurred in patients who experienced nausea, vomiting, or diarrhea, leading to volume depletion. Monitor renal function when initiating or escalating doses of Wegovy™ in patients reporting severe adverse gastrointestinal reactions and in patients with renal impairment reporting any adverse reactions that could lead to volume depletion.
- Hypersensitivity: Serious hypersensitivity reactions (e.g., anaphylaxis, angioedema) have been reported with semaglutide. If hypersensitivity reactions occur, discontinue use of Wegovy™, treat promptly per standard of care, and monitor until signs and symptoms resolve. Use caution in a patient with a history of anaphylaxis or angioedema with another GLP-1 receptor agonist.
- Diabetic Retinopathy Complications in Patients with Type 2 Diabetes: In a trial of patients with type 2 diabetes, diabetic retinopathy was reported by 4.0% of Wegovy™ patients and 2.7% of placebo patients. Rapid improvement in glucose control has been associated with a temporary worsening of diabetic retinopathy. Patients with a history of diabetic retinopathy should be monitored for progression of diabetic retinopathy.
- Heart Rate Increase: Mean increase in resting heart rate of 1 to 4 beats per minute (bpm) were observed in Wegovy™ patients compared to placebo in clinical trials. More Wegovy™ patients compared with placebo had maximum changes from baseline of 10 to 19 bpm (41% versus 34%) and 20 bpm or more (26% versus 16%). Monitor heart rate at regular intervals and instruct patients to report palpitations or feelings of a racing heartbeat while at rest. If patients experience a sustained increase in resting heart rate, discontinue Wegovy™.
- Suicidal Behavior and Ideation: Suicidal behavior and ideation have been reported in clinical trials with other weight management products. Monitor patients for depression, suicidal thoughts or behavior, and/or any unusual changes in mood or behavior. Discontinue Wegovy™ in patients who experience suicidal thoughts or behaviors and avoid in patients with a history of suicidal attempts or active suicidal ideation.

Adverse Reactions
- The most common adverse reactions reported in ≥5% of patients treated with Wegovy™ are nausea, diarrhea, vomiting, constipation, abdominal pain, headache, fatigue, dyspepsia, dizziness, abdominal distention, eructation, hypoglycemia in patients with type 2 diabetes, flatulence, gastroenteritis, and gastroesophageal reflux disease.

Drug Interactions
- The addition of Wegovy™ in patients treated with insulin has not been evaluated. When initiating Wegovy™, consider reducing the dose of concomitantly administered insulin secretagogues (such as sulfonylureas) or insulin to reduce the risk of hypoglycemia.
- Wegovy™ causes a delay of gastric emptying and has the potential to impact the absorption of concomitantly administered oral medications. Monitor the effects of oral medications concomitantly administered with Wegovy™.

Use in Specific Populations
- Pregnancy: When pregnancy is recognized, discontinue Wegovy™. Discontinue Wegovy™ in patients at least 2 months before a planned pregnancy.

Click here to see the Prescribing Information, including Boxed Warning.

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Liquid Sucrose Consumption Promotes Obesity and Impairs Glucose Tolerance Without Altering Circulating Insulin Levels

Susan J. Burke1, Heidi M. Batdorf1, Thomas M. Martin1, David H. Burk1, Robert C. Noland1, Christopher R. Cooley2, Michael D. Karlstad2, William D. Johnson1, and J. Jason Collier 1

Objective: Multiple factors contribute to the rising rates of obesity and to difficulties in weight reduction that exist in the worldwide population. Caloric intake via sugar-sweetened beverages may be influential. This study tested the hypothesis that liquid sucrose intake promotes obesity by increasing serum insulin levels and tissue lipid accumulation.

Methods: C57BL/6J mice were given 30% sucrose in liquid form. Changes in weight gain, body composition, energy expenditure (EE), and tissue lipid content were measured.

Results: Mice drinking sucrose gained more total body mass (TBM), had greater fat mass, and displayed impaired glucose tolerance relative to control mice. These metabolic changes occurred without alterations in circulating insulin levels and despite increases in whole body EE. Lipid accrued in liver, but not skeletal muscle, of sucrose-consuming mice. Oxygen consumption (VO2) correlated with fat-free mass and moderately with TBM, but not with fat mass. ANCOVA for treatment effects on EE, with TBM, VO2, lean body mass, and fat-free mass taken as potential covariates for EE, revealed VO2 as the most significant correlation.

Conclusions: Weight gain induced by intake of liquid sucrose in mice is associated with lipid accrual in liver, but not skeletal muscle, and occurs without an increase in circulating insulin.


Introduction

Obesity is a continually increasing major public health problem worldwide (1) and is a major comorbidity for other disorders, including cancer, diabetes, heart disease, and hypertension (2). Higher BMI values disproportionately contribute to rising health care costs (3). Multiple factors influence conditions of overweight and obesity, including genetic predisposition, sedentary lifestyles, reduced levels of physical activity, and food choices (4).

Sugar-sweetened beverages (SSBs) have historically been controversial as risk factors for, or direct contributors to, the development of obesity because of arguments over study design and other experimental issues (see (5,6) and references therein). Critical compilation of data from more recent studies has concluded that SSBs influence or exaggerate the effect of the principal factors leading to conditions of overweight and obesity (7,8). Mono- and disaccharides constitute the major sweeteners in foods and beverages that, when ingested in either liquid or solid form, influence weight gain and metabolic disease onset (5,9,10). Sucrose is a disaccharide that supplies glucose and fructose in equimolar amounts once digested. Glucose metabolism is subject to regulation through glycolytic pathways, including allosteric regulation by the enzyme 6-phosphofructo-2 kinase isoform 1, whereas fructose is phosphorylated by fructokinase and cleaved into trioses (e.g., by aldolase B), thus bypassing this regulation (11).

Sucrose is a very rewarding stimulus to many organisms and therefore is often preferentially sought out (12). Consistent with this idea, the intake of beverages containing sucrose or other related sweeteners has increased dramatically during the past 50 years (7). However, within 10 weeks, sucrose intake was shown to worsen the metabolic profile of human subjects with overweight (13). In addition, obesogenic diets promote hyperinsulinemia, which could be a contributor to insulin resistance, obesity, or both (14,15).

Because most animal models of obesity exhibit hyperinsulinemia (16), elevations in circulating insulin could be either a driver or a...
consequence of obesity. Genetic reduction of insulin genes in mice has revealed that abundant quantities of insulin are indeed required to support weight gain and increased adiposity during high-fat feeding (17,18). However, obesity associated with high-fat diet could be distinct from obesity driven by overconsumption of other macronutrients (e.g., sucrose); thus, macronutrient imbalance may be sensed by specific endocrine mechanisms. Fibroblast growth factor 21 (FGF21) is a protein hormone with wide effects on metabolic outcomes and is thus poised to participate in nutrient sensing (19). Indeed, there is precedent for elevations in FGF21 during consumption of a low-protein diet, consistent with a role for macronutrient sensing (20). Because the Fgf21 gene is also activated in response to elevations in dietary carbohydrate (21), fasting (22), or glucocorticoids (23), it is reasonable to postulate that a variety of homeostatic mechanisms are activated by dietary alterations. Our studies herein reveal that C57BL/6J mice consuming liquid sucrose gain weight without an increase in circulating insulin and display many common features with human subjects who have overweight or obesity.

Methods

Animals and reagents

Sixteen male C57BL6J (stock no. 000664) mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) at 8 weeks of age and allowed free access to Lab Diet 5015 standard nonpurified diet (catalog no. 0001328). For comparison with a separate model of obesity, sixteen male db/db mice (stock no. 000697) were acquired from the Jackson Laboratory at 8 weeks of age and housed until 14 weeks of age. All mice were allowed to acclimate to the photoperiod (12 hours light/12 hours dark) and temperature conditions (22°C ± 1°C) for at least 7 days to provide time for normalization of physiological parameters due to transport (24). Mice offered a 30% sucrose solution show a strong preference to this liquid over water (25). Blood glucose was determined by weekly tail vein sampling using a 25-gauge needle. Body weight and body composition (by time-domain nuclear magnetic resonance) were also measured weekly. Food and liquid intake was measured daily by weighing the amount provided and subtracting the mass of the amount consumed over a 24-hour period. Measurements were taken at the same time each day for 2 weeks. Following a 4-hour fast, animals were sedated by carbon dioxide exposure and then euthanized by cervical dislocation. For measurements of energy expenditure (EE), activity, and sleep time, mice were 11 weeks of age when acclimated to the training cages for 1 week. Thus, they were 12 weeks of age during the actual measurements. In the metabolic cage, corn cob bedding was included and there was an intake manifold (a small metal tube that runs along the perimeter of the cage to pull air). The training cage was exactly the same (bedding, dimensions, etc.) as the testing cage, minus the manifold on the perimeter of the cage. Mice were single housed in the training cages and also in the metabolic cages during measurements. Animals were allowed continued free access to either standard drinking water (control group) or 30% sucrose (sucrose group) during metabolic cage time. All procedures were approved by the University of Tennessee (protocol 2171-0216) and Pennington Biomedical Research Center Institutional Animal Care and Use Committee (protocol 972).

Glucose tolerance tests

Glucose tolerance measurements were taken at 3 and 6 weeks into the 12-week study. Animals in the 30% sucrose group were switched to standard drinking water overnight prior to glucose tolerance testing, and the next morning, both groups were placed in clean cages and fasted for 4 hours before beginning the glucose tolerance test protocol. A 20% glucose solution was administered at a concentration of 2.5 g/kg body weight via intraperitoneal injection. Blood glucose was read prior to injection using a Breeze2 glucometer (Bayer) using tail vein blood (time 0 minutes) and again at 20, 40, 60, and 120 minutes after intraperitoneal glucose injection.

Reverse transcription-polymerase chain reaction, cDNA synthesis, and mRNA analysis

Total RNA was isolated from tissues using the guanidinium thiocyanate method (26), complementary DNA (cDNA) was synthesized from total RNA, and mRNA integrity was analyzed, with mRNA transcripts measured using the Carbohydrate Metabolism and Regulation of Lipid Metabolism PrimerPCR plates (M384) from Bio-Rad. This strategy allows for objective screening of key genes encoding enzymes linked with control of intermediary metabolism. All other primers used were designed using Primer3Plus software, version 2.4.2 (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and are available in Supporting Information Table S1. Normalization procedures to housekeeping control gene ribosomal protein 9 (Rps9) have been described (27). Expression was deemed not detectable and given the label “N.D.” if no expression over baseline was detected by 40 cycles. Kits and reagents used, and protocols for procedures, have been previously described (28).

Glycogen and acyl glycerol assays

Total acyl glycerol was measured in rectus abdominis (RA), mixed gastrocnemius (MG), and liver. Glycogen levels were assessed in liver and RA tissues. Reagents and protocols for both procedures have been described in detail elsewhere (15,28).

Serum factors

The following kits were used to measure serum hormones: Mouse/Rat Leptin Quantikine (catalog no. MOB00) and Mouse/Rat FGF21 Quantikine (catalog no. MF2100) enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems and the Mouse Insulin ELISA kit (catalog no. 10-1247-01) from Merckodia, which were used according to the manufacturers’ suggested protocols.

Pancreatic islet histology

After tissue collection in neutral-buffered formalin, pancreata were embedded in paraffin. Five-micrometer sections were cut and placed onto positively charged slides. Insulin-positive area (determined in square microns) was measured by immunohistochemistry. A complete description of the methods, equipment, and reagents used for detection of insulin and quantification of insulin-positive area have been provided previously (29).

Statistical analysis

One-way analysis of variance (ANOVA) was used when comparing three or more groups, repeated-measures ANOVA was used for comparing continuous variables, Pearson correlations were calculated to assess linear associations between two variables, and t tests were performed to compare two groups with respect to their outcome means. These measures were conducted using GraphPad Prism software version 6.0.
GraphPad Prism was used to calculate the area under curve (AUC) for the glucose tolerance tests. Prism computes AUC using the trapezoid rule (30). Corresponding $P$ and $r$ values for the respective experiments are given in figures or figure legends. Analysis of covariance (ANCOVA) was performed to assess the impact of oxygen consumption ($V_{O_2}$), total body mass (TBM), fat mass (FM), and fat-free mass (FFM) as covariates on EE. Correlation analyses assessed the magnitude of statistical associations among the covariates. Statistical models were employed to evaluate statistical significance of differences between sucrose and water with respect to EE, $V_{O_2}$, TBM, FM, FFM, and lean mass (LM). The SAS statistical software package, version 9.4, was used to perform the ANCOVA analytic calculations. The AUC was significantly different for both raw (Figure 2F; $P < 0.05$) and normalized values (Figure 2H; $P < 0.001$).

**Results**

**Sucrose intake in liquid form alters body composition and glucose tolerance**

Free access to a liquid 30% sucrose solution led to obesity in male C57BL/6J mice (Figure 1). We found that mice, which were weight matched at the beginning of the study, began to develop significantly increased body mass 2 weeks after *ad libitum* consumption of liquid sucrose (Figure 1A). This increase in body mass was sustained over the course of the 12-week period (Figure 1A), with mice consuming sucrose displaying 39.6% more TBM over the control group (Figure 1B). Mice drinking water ate similar amounts of solid food (3.51 g/d) compared with mice drinking sucrose (3.35 g/d). However, mice offered liquid sucrose consumed more liquid (10.26 g/d) when compared with the water control group (6.11 g/d), leading to greater caloric intake in the sucrose group (17.80 kcal/d) relative to the control group (12.97 kcal/d).

LM was enhanced as early as 2 weeks (Figure 1C) in the sucrose group, with a 40.3% gain over control by the end of the study (Figure 1D). In addition, FM was elevated within 2 weeks after liquid sucrose consumption (Figure 1E), with a 51% increase in total FM in the sucrose group relative to the control mice (Figure 1F). The expansion of total FM was consistent with an increase in epididymal white adipose tissue (eWAT) weight after 4 weeks of continuous sucrose intake (Figure 1G). We note that there was no significant change in body length (measured nose to anus) in response to sucrose consumption (Figure 1H).

Three weeks after sucrose consumption, glucose tolerance was not significantly altered (Figure 2A-2D). However, by 6 weeks, the mice consuming sucrose displayed clear alterations in glucose tolerance (Figure 2E, raw data, and Figure 2F, normalized data). The AUC was significantly different for both raw (Figure 2F; $P < 0.05$) and normalized values (Figure 2H; $P < 0.001$).

**Elevations in leptin and FGF21, but no change in serum insulin, in response to liquid sucrose consumption**

During the first week of sucrose drinking, there was no detectable difference in blood glucose between the mice (Figure 3A). However, by the end of the study, the mice consuming sucrose displayed a trend toward increased blood glucose (Figure 3A; 12-week water vs. sucrose: $P = 0.07$). Serum levels of insulin also remained similar between groups (Figure 3B). Leptin was elevated during the first week on sucrose (Figure 3C), which was consistent with the early increase in FM (Figure 1E-1G). However, by the end of the 12-week study, leptin levels were similar between the sucrose and control groups (Figure 3C). There was a trend toward increased islet fraction (insulin-positive area/pancreas area; Figure 3D; $P = 0.11$), consistent with a modest increase in insulin-positive area in response to sucrose (Figure 3F). FGF21, a hormone known to be induced by carbohydrate *in vitro* (21), was elevated *in vivo* in response to sucrose intake (Figure 3E).

**Sucrose ingestion increases respiratory quotient, EE, and caloric intake but does not impact activity or sleep time**

The increase in body mass observed in mice consuming sucrose (Figure 1) was not due to a decrease in total activity measured...
during metabolic cage analyses (Figure 4A). Additionally, average total sleep time was not different in mice drinking sucrose versus the control group during the study (Figure 4B). As expected, the respiratory quotient (RQ) was higher in mice consuming sucrose (Figure 4C-4D), reflecting an increase in carbohydrate usage. Despite no differences in physical activity, raw EE (average output per mouse) was increased by 22% in the group drinking sucrose relative to mice drinking water (Figure 4E-4F). EE correlated with TBM ($r = 0.91$, $P < 0.0001$; Figure 5A), FFM ($r = 0.94$; $P < 0.0001$; Figure 5C), and FM ($r = 0.77$; $P < 0.001$; Figure 5E). By contrast, VO2 increased moderately as a function of TBM ($r = 0.60$; $P < 0.05$; Figure 5B) and increased strongly with FFM ($r = 0.75$; $P < 0.001$; Figure 5D) but not with FM ($r = 0.31$; $P = 0.25$; Figure 5F). ANCOVA for treatment effects on EE, with TBM, VO2, and FFM taken as potential covariates for EE, revealed that VO2 was the most influential single covariate (Supporting Information Table S2). When the treatment means for EE were covariate adjusted for VO2, the EE mean for sucrose was significantly elevated ($P < 0.025$).

**Figure 2** Chronic consumption of sucrose impairs glucose tolerance in C57BL/6J mice. Glucose tolerance tests (GTTs) were performed at 3 and 6 weeks into the study period. Data are represented as (A,E) raw values or (C,G) normalized data in which the initial time 0 value is set to 100% for both groups. Area under curve (AUC) calculations for GTTs performed at (B,D) 3 and (F,H) 6 weeks. Data are shown as means ± SEM. n = 8 per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus respective controls.

**Figure 3** Sucrose consumption increases circulating leptin and fibroblast growth factor 21 (FGF21), but not insulin. (A) Blood glucose, (B) serum insulin, and (C) serum leptin were measured in male C57BL/6J mice after 1, 4, or 12 weeks in control (white bars) versus sucrose (black bars). (D) Ki (black bars) circulating levels of FGF21, and (F) insulin-positive area were assessed. Data are represented as means ± SEM. n = 4 per group (1-week study) and n = 8 per group (12-week study). *$P < 0.05$, **$P < 0.01$. 

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additional covariate, each covariate was significantly correlated with EE. Using linear regression models, we determined that the proportionate amount of total variability in EE that could be attributed to each of the covariates was 0.827 (TBM), 0.590 (FM), and 0.882 (FFM), respectively. However, when VO₂ and TBM were simultaneously held constant, the other covariates being considered were not significant contributors to the prediction of EE (Supporting Information Table S2).

Lipid, but not glycogen, storage increases in liver during sucrose consumption

Total acyl glycerol accumulation was augmented in liver tissue starting 1 week after sucrose consumption (Figure 6A) and remained elevated over control at 12 weeks (Figure 6A). By contrast, liver glycogen storage was not impacted by either acute (1 week) or chronic (12 weeks) sucrose consumption (Figure 6B). Several genes involved in

Figure 4 Sucrose increases energy expenditure (EE) and respiratory quotient (RQ) but does not alter activity or sleep time. Mice were acclimated to drinking water or a 30% sucrose solution for 1 week prior to placement in metabolic cages. (A) Total activity and (B) mean sleep time during access to either water (white bars) or sucrose (black bars). (C) RQ at daily intervals showing light cycle (white) and dark cycle (gray). (D) Energy expenditure (EE) at daily intervals showing the light and dark cycles. (E) Average EE over a 5-day period. *P < 0.001 versus water.

Figure 5 Increased oxygen consumption (VO₂) in response to sucrose intake correlates strongly with fat-free mass.

Energy expenditure (EE) expressed as a function of (A) total body mass (TBM), (C) fat-free mass (FFM), and (E) fat mass (FM) in mice consuming either water (gray circles) or sucrose (black squares). VO₂ shown in relationship to (B) TBM (epididymal white adipose tissue), (D) FFM, and (F) FM. *P < 0.001 versus water.
carbohydrate processing were expressed more robustly at both acute and chronic time points (Figure 6C-6D). For example, aldolase B (AldoB) was elevated more than threefold in the sucrose group relative to the control group after 1 week (Figure 6C). AldoB encodes the liver form of the enzyme, which catalyzes the reversible cleavage of fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate as well as the bidirectional fragmentation of fructose 1-phosphate into glyceraldehyde and dihydroxyacetone phosphate. The AldoB gene remained highly expressed at 12 weeks post sucrose (>4-fold; Figure 6D). In addition, aldolase C (AldoC), which is normally expressed in the central nervous system, was upregulated approximately 19-fold by 12 weeks (Figure 6D), which likely reflects the need to process the abundance of carbohydrates being ingested.

Consistent with carbohydrate conversion to lipid as a storage form, the genes encoding acetyl-coenzyme A carboxylase (Acaca), fatty acid synthase (Fasn), and microsomal triglyceride transfer protein (Mttp) were all elevated in mice consuming sucrose relative to controls (Figure 6E). Genes in the serpin class encode protease inhibitors that regulate a variety of biological processes, including inflammation. We found that the serpin family A member 12 (SerpinA12, also known as vaspin) gene was selectively expressed more than 12-fold in the livers of mice consuming sucrose (Figure 6F).

Sucrose enhances expression of genes associated with lipid metabolism in eWAT but does not promote lipid or glycogen storage in skeletal muscle

Because of the increase in LM observed in response to sucrose consumption (Figure 1C), we examined both lipid and glycogen content in skeletal muscle. Using RA and MG, we found that although MG contained more total acyl glycerols when compared with RA, neither RA nor MG displayed elevated triglycerides in response to sucrose intake (Figure 7A). Although glycogen content increased with age in RA muscle of control mice (compare 1 week with 12 weeks; white bars), the group drinking sucrose did not accumulate glycogen over time (Figure 7B). Although surprising at first glance, these results in mice are similar to those observed in humans, in whom muscle glycogen is reduced for subjects with obesity and type 2 diabetes (31). We also observed that there were minimal changes in expression of glycolytic enzyme genes in RA muscle after 1 week on sucrose (Figure 7C). However, by 12 weeks, there was a marked increase in genes encoding glycolytic enzymes, including upregulation of phosphoglycerate mutase 1 (Pgam1), pyruvate kinase, muscle (Pkm), and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) (Figure 7D).

When examining the eWAT from mice consuming sucrose versus controls, most genes encoding glycolytic enzymes were either unchanged or trending toward decreased expression (Figure 7E-7F). By contrast, several genes that regulate lipid metabolism were distinctly upregulated. For example, expression of carnitine palmitoyltransferase-1b (Cpt1b), which encodes an enzyme involved in fatty acid oxidation, was increased threefold in eWAT in response to sucrose (Figure 7G). However, fatty acid binding protein 7, brain (Fabp7) and fatty acid binding protein 1, liver (Fabp1) were selectively expressed at higher levels (≥5-fold) in mice ingesting sucrose relative to controls (Figure 7G).

Differential pattern of gene expression in inguinal white adipose tissue from mice consuming sucrose when compared with inguinal white adipose tissue from genetically obese db/db mice

We next compared expression of the cell death-inducing DNA fragmentation factor, alpha subunit-like effector A (Cidea) and uncoupling
protein 1 (Ucp1) genes, which often indicate browning of white adipose tissue, using the inguinal white adipose tissue (iWAT) depots from two different models of obesity: mice consuming sucrose for 12 weeks in liquid form versus db/db mice, a genetic model of obesity produced by a defect in leptin receptor signaling. We found that the expression of Ucp1 and Cidea genes were elevated 28- and 15-fold, respectively, in response to sucrose consumption (Figure 8A). By contrast, expression of Ucp1 was undetectable in iWAT from db/db mice (Figure 8B). Although Cidea was clearly increased in mice drinking sucrose, expression of this gene decreased in db/db mice relative to the lean controls (Figure 8B). Next, we observed that expression of nucleotide-binding oligomerization domain containing 1 (Nod1) was reduced by 89% in mice consuming sucrose (Figure 8C). This is interesting because NOD1 restricts adipocyte differentiation (32); thus, a decrease in its expression would be expected to allow the expansion of adipose tissue, consistent with what was observed during sucrose intake (Figure 1). We observed that neither db/db mice nor the lean control mice (db/+) expressed appreciable amounts of Nod1 transcripts in iWAT (Figure 8D).

Expression of Adipoq, encoding the hormone adiponectin, was decreased in both the sucrose model of obesity and in db/db mice (Figure 8C-8D). In addition, serum amyloid A 3 (Saa3) was expressed at lower levels in sucrose-consuming mice (Figure 8E).

Figure 7 Sucrose increases expression of genes linked with carbohydrate metabolism in skeletal muscle and expression of genes controlling lipid metabolism in epididymidal white adipose tissue (eWAT). (A) Total acyl glycerol and (B) glycogen content was measured in the rectus abdominis and mixed gastrocnemius (MG) muscle depots from male C57BL/6J mice. Expression of genes involved in carbohydrate metabolism was determined in (C,D) rectus muscle and (E,F) eWAT. Data in panels C-F are represented as a ratio of expression of the 30% sucrose group versus water group (dashed line indicates expression of water control group). (G) Total mRNA abundance of genes regulating lipid metabolism was studied in the eWAT from mice on an oral 30% sucrose regimen. Data are shown as means ± SEM. n = 8 per group (A,B,G) or n = 3 per group (C-F). **P < 0.01 versus respective controls.

Figure 8 Distinct differences in gene expression profiles in inguinal white adipose tissue (iWAT) from obese db/db mice compared with C57BL/6J mice made obese by liquid sucrose intake. Relative mRNA abundance of genes expressed in iWAT from either C57BL/6J mice drinking either 30% sucrose or water (A,C,E,G,I) versus 14-week-old db/+ and db/db mice (B,D,F,H,J). Data are plotted as means ± SEM. n = 8 per group. *P < 0.05, **P < 0.01, ***P < 0.001 versus respective controls. N.D. = not detectable.
but was strikingly increased in expression in db/db mice (Figure 8F). In addition, the macrophage marker Cd68 was expressed at lower levels in obesity driven by sucrose (Figure 8E) but was unchanged in db/db mice relative to controls (Figure 8F).

Adipose tissue expression of vascular endothelial growth factors (VEGFs) modulates insulin resistance during high-fat feeding (33). Indeed, the metabolic benefits of intermittent fasting require VEGF, which may be in part through effects on macrophage polarization (34). Weight gain induced by sucrose intake was associated with a 93% decrease in expression of Vegfa and an 88% reduction in Vegfb mRNA levels (Figure 8G). In adipose tissue of db/db mice (relative to the lean controls), the expression of Vegfa increased by 2.3-fold, whereas Vegfb transcripts declined by 56% (Figure 8H).

Diacylglycerol acyltransferases, encoded by the Dgat1 and Dgat2 genes, catalyze the final step in triglyceride synthesis. We found that expression of Dgat1 and Dgat2 were reduced by 92% and 97%, respectively, in the iWAT from mice drinking sucrose (Figure 8I). However, the expression of these genes was unchanged between lean db/+ and obese db/db mice (Figure 8J).

Discussion

Caloric overload in its various forms (e.g., high-fat diet, SSBs), coupled with sedentary lifestyle, are major contributors to the rising rates of obesity. Indeed, nutrient excess from SSBs has increased substantially over the past 50+ years (7), likely contributing to the rising prevalence of metabolic disease (8). In the present study, we provided male C57BL/6J mice with free access to a 30% sucrose solution to investigate the specific metabolic consequences that occur in response to liquid sugar intake. Because all mice had access to the same solid chow diet, major metabolic differences between groups were able to be directly compared. One of the key findings herein is that changes in body composition precede changes in glucose tolerance.

Humans with a high 24-hour RQ are more likely to show weight gain than those with a low RQ (35). Consistent with these results, we found that C57BL/6J mice consuming liquid sucrose consistently had higher RQ values (Figure 4C-4D) and that these mice gained more weight (Figure 1). However, elevated levels of insulin were clearly not required to achieve this enhanced body mass in response to liquid sucrose consumption (Figure 3B). These data provide an intriguing difference between obesity induced by a high-fat diet, in which insulin levels rise (15,36), and adiposity driven by liquid sucrose. Thus, whether insulin is simply permissive for weight gain or is a direct contributor to weight gain may be dependent on dietary context (i.e., precise macronutrient intake).

When adiposity decreases, there are corresponding reductions in circulating insulin (37). Interestingly, mice with genetic deletion of multiple copies of the insulin gene resist weight gain on a high-fat diet (17), indicating that a threshold level of insulin may be required to support adiposity and elevations in TBM in response to excess dietary lipid. Because elevations in circulating insulin are not required to promote weight gain or deposition of adipose tissue during ad libitum access to liquid sucrose, we interpret these data to indicate that excess carbohydrate intake is a particularly potent factor supporting weight gain. Thus, under certain conditions (e.g., free consumption of SSBs), the amount of basal insulin present in circulation is likely sufficient to route the excess substrate into lipid storage and/or to adequately suppress fatty acid oxidation in favor of carbohydrate oxidation, resulting in higher RQ values and subsequent weight gain. A separate but not mutually exclusive possibility is that calories consumed in liquid versus solid form may elicit distinct endocrine responses in mice, consistent with observations in humans (38).

Intriguingly, glycogen content of liver (Figure 6B) and muscle (Figure 7B) was not elevated by sucrose consumption, whereas liver lipid content was increased (Figure 6A). Expression of the SerpinA12 gene, which is linked with obesity and type 2 diabetes (39), was upregulated in livers of mice consuming sucrose (Figure 6F). Although insulin-positive area was modestly enhanced by sucrose consumption (Figure 3F), islet fraction was unaltered (Figure 3D). By contrast, excess lipid in the diet promotes enlarged pancreatic β-cell mass, generally resulting from enhanced β-cell proliferation within the islets (40).

Perhaps greater circulating FGF21 levels serve as an insulin “sensitizing” signal or as a feedback loop to prevent excess weight gain by increasing EE (Figure 4E). Indeed, augmented FGF21 in circulation promotes browning of white adipose tissue (see reference (41) and Figure 8A), which often correlates with enhanced EE (see Figure 4E-4F and reference (42)). Importantly, our results using C57BL/6J mice consuming sucrose are analogous to human studies showing that EE increased specifically in subjects ingesting a sucrose-rich diet (43). However, two limitations of our study are that we did not use clamp techniques to determine whether the lipid accumulation in liver prevents the normal suppression of hepatic glucose production by insulin, and we did not study obesity driven by sucrose in female mice. These are important considerations for future studies.

In summary, sucrose intake promotes weight gain and adiposity with distinct metabolic differences when compared with the db/db mouse model of obesity. From a translational perspective, the data in this study agree with human studies in several aspects. First, sucrose intake rapidly promotes greater weight gain and increased FM in mice (Figure 1) and humans (13). Second, EE is greater in mice (Figure 4E) and humans consuming sucrose (43). Third, elevations in RQ are associated with weight gain in mice (Figure 4C) and humans (35). Fourth, sucrose promotes lipid accumulation in liver of mice (Figure 6A), rats (44), and humans (45). In summary, we conclude that male C57BL/6J mice consuming liquid sucrose provide a model of obesity and glucose intolerance with key physiological features that are relevant to human metabolic disease.

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References


