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Comparable metabolic effects of isocaloric sucrose and glucose solutions in rats

Michael D. Kendig[a]*, Sarah I. Martire[a], Robert A. Boakes[a], & Kieron B. Rooney[b]

[a] School of Psychology, University of Sydney, NSW, 2006, Australia

[b] Faculty of Medicine and Health, University of Sydney, NSW, 2006, Australia

*Corresponding author:

Dr. Michael D. Kendig.

Present address:

Wallace Wurth Building (Level 3 East)

School of Medical Sciences, UNSW Sydney

NSW 2052

Australia

Email: m.kendig@unsw.edu.au

Phone: (612) 9385 1621

Dr. Sarah I. Martire: sarah.martire@sydney.edu.au

Professor Robert A. Boakes: bob.boakes@sydney.edu.au

Associate Professor Kieron B. Rooney: kieron.rooney@sydney.edu.au

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Abstract

Much of the global increase in sugar intake is attributable to rising consumption of sugar-sweetened beverages (SSBs). Because people compensate poorly for liquid calories, SSB consumption increases total energy intake, raising the risk of harmful metabolic effects in addition to possible effects of sugars *per se*. Glucose and fructose, the constituent sugars in sucrose, can exert distinct effects on metabolism and also differ in their satiating properties, suggesting that compensation for the calories in these sugars may also vary. In light of claims that the fructose within sucrose is particularly harmful, the present study compared the effects of giving rats access to either a sucrose or an isoenergetic glucose solution. Adult male rats were fed standard chow and water supplemented with 95 ml of 10% glucose (Glucose group; $n = 10$), 9% sucrose solution (Sucrose group; $n = 10$) or water only (Control group; $n = 10$) daily for 7 weeks. Sugar-fed groups had higher total energy intakes than the Control group, but the extent of this incomplete compensation did not vary between Sucrose and Glucose groups. In a short-term compensation test, sugar groups were less sensitive to the effects of a sweet pre-meal, with no differences between the Glucose and Sucrose groups. Relative to water, both sugars reduced insulin sensitivity after 4 weeks on the diets and elevated fat mass at 7 weeks. Results suggest that sucrose and glucose induce comparable metabolic impairments and alter the homeostatic regulation of food intake even under conditions where daily access is capped.

Keywords: glucose; sucrose; metabolism; compensation; rats.

1. Introduction

High dietary sugar intake is associated with an increased risk of obesity [1, 2], metabolic disorders [3] and cardiovascular disease [4]. Sugar-sweetened beverages (SSBs) make a leading contribution to added sugar intake in children, adolescents and adults [5]. Relative to sugary foods, SSBs are less satiating [6] and may increase the risk of developing the metabolic syndrome [7].

Studies modeling SSB intake in rats and mice have shown that supplementing a standard chow diet with sugar solutions increases total energy intake, body weight gain and adiposity, and induces insulin resistance, even when provided as a 10% solution that approximates the concentration of most commercially available SSBs [8-12]. Solid diets high in sugar are known to reduce lifespan and reproductive fitness in mice [13] and induce hepatic insulin resistance in rats [14].

Some accounts attribute the adverse effects of sucrose intake to its fructose component, which is thought to increase hepatic fat and plasma triglycerides when consumed in excess [15, 16]. For example, a 10-week intervention study in overweight and obese adults found that fructose- but not glucose-containing drinks impaired insulin sensitivity and increased visceral fat [17], while a within-subjects crossover study comparing isocaloric meals high in fructose or glucose [18] showed that the fructose meal led to smaller excursions in postprandial glucose, insulin and leptin, and smaller reductions in plasma ghrelin. Fructose solution supplementation can induce aspects of the metabolic syndrome in rodents (see [19] for review) and direct comparisons with glucose have found that chronic consumption of fructose solutions leads to poorer metabolic outcomes in liver, white adipose tissue and skeletal muscle [20], heart [21] and brain [22].

The effects of fructose and glucose on satiety and food intake are complex: while fructose appears to promote greater satiety over the short-term [23, 24], some studies report a greater reduction in food intake following pre-meals with a higher glucose:fructose ratio [25] and stronger flavour preferences can be conditioned to glucose than fructose [26] due to distinct post-ingestive effects of the two sugars [27]. The effects of substituting fructose for other sugars were discussed in a systematic review and meta-analysis by Evans and colleagues [28]. However, it is unclear whether the ability to compensate for novel energy-dense foods is altered by long-term consumption of a diet high in fructose-free versus fructose-containing sugars.

Others argue that the detrimental metabolic effects of sugar are not attributable to fructose *per se* but instead result from increased total energy intake (e.g. [29]). Thus, because ~10% sugar solutions are highly palatable, they are consumed avidly, leading to metabolic complications when not offset by reduced intake elsewhere in the diet. A prediction stemming from this account is that other palatable solutions that serve to increase energy intake will induce comparable effects as sucrose, regardless of whether they contain fructose. In a previous study [30], we compared 11 weeks' *ad-libitum* access to sucrose or an isoenergetic maltodextrin solution, which is very palatable for rats [31], and found comparable effects on body weight, fasting blood glucose and retroperitoneal fat. Both solutions impaired hippocampal-based spatial memory relative to chow-fed controls, suggesting fructose is not needed to induce metabolic and cognitive impairments in rats [30].

The present study extended this line of research by directly comparing glucose with sucrose, following unpublished work from our labs where we observed that, unlike sucrose, rats exposed to 10% glucose solution compensated perfectly for liquid calories and exhibited good glycemic control in an oral glucose tolerance test. Following pioneering work by Richter [32], only a few

studies have directly compared sucrose and glucose solutions. Kanarek and Orthen-Gambill [33] fed male rats standard chow and water supplemented with a 32% glucose, 32% sucrose, or 32% fructose solution for 50 days, while a control group was given only chow and water. Sucrose- and fructose-fed groups exhibited the greatest energy intake, weight gain and glucose intolerance, whereas the glucose-fed group were normoglycemic and exhibited a smaller increase in body weight relative to controls. However, comparisons between the Sucrose and Glucose groups were complicated by the difference in their consumption of the solutions, with greater intake by the sucrose than glucose group.

Sclafani [34] compared the effects of access for 40 days to 32% sucrose, 32% glucose or 32% maltodextrin (Polycose) solutions in female rats. Sucrose and Glucose groups derived approximately 60% of calorie intake from their sugar solutions, but did not differ in weight gain, total energy intake, fat or fasting plasma insulin and glucose. In a short-term study where sugar solutions were the only fluids available, Kazumi, Vranic, and Steiner [35] found no differences in intake between groups of rats fed 10% glucose, sucrose, or fructose solutions for 2-weeks. While sucrose and glucose groups did not differ on any measure, all sugar-fed groups were hyperinsulinemic and hyperglycemic relative to controls, with elevated triglyceride concentrations only in the fructose group. Lindqvist, Baelemans, and Erlanson-Albertsson [36] compared water-only control rats to groups fed 23% solutions of glucose, sucrose, or fructose for 2-weeks. Sugar-fed rats exhibited greater weight gain and poorer metabolic outcomes relative to controls, with few differences between the sugar groups, and all exhibiting partial compensation for the liquid calories by consuming less chow than controls [36]. In summary, the few direct comparisons between sucrose and glucose have either used concentrated solutions [33, 34] or tested over relatively short periods in forced-intake models [35, 36].

The present study compared rats' short- and long-term compensation for sucrose and glucose solutions at the concentration of SSBs consumed by people, and under conditions where consumption of the solutions was equated. The primary outcome measures were chow intake, which was predicted to be lower in the Glucose than in the Sucrose group, thus indicating greater compensation for the energy in their sugar drinks; and glucose tolerance, which was predicted to be impaired only in the Sucrose group. Our predictions for chow intake and glucose tolerance were derived from preliminary, unpublished data obtained from our laboratory. In addition to long-term compensation – measured by home-cage chow intake – we tested rats' ability to compensate for a novel, sweet pre-meal over a 24-h period. Short-term object and place recognition memory were assessed after 5-6 weeks of the diet, and fat mass was evaluated at endpoint.

2. Method

2.1. Animals and housing

Thirty 7-week old male Sprague Dawley rats were purchased from Animal Resource Centre, Perth, Australia. On arrival rats were group-housed (n = 5/cage) in large plastic cages (62 x 40 x 26 cm high) within a temperature- and humidity-controlled room (22-24°C and 40-60% humidity) on a normal 12 h light/dark cycle (lights on at 0700 hrs). After four days of acclimation and handling, rats were transferred to individual plastic cages (46 x 27 x 32 cm high) and placed on a reverse 12-h dark/light cycle (lights off 0930 – 2130 hrs). At this time they were randomly allocated to Glucose, Sucrose, or Control groups (each n = 10). The experiment began after four days of acclimation to the new lighting schedule. All procedures were approved by the Animal Ethics Committee at the University of Sydney (L29/8-2010/3/5354) and were conducted

in accordance with the Australian code for the care and use of animals for scientific purposes 8th edition (2013).

2.2. Diet intervention (Days 1-49)

Standard chow (Specialty Feeds, 14.2 kJ/g; 20% protein, 4% fat, 60% carbohydrate; http://www.specialtyfeeds.com) and water were available *ad-libitum* throughout the 7-week diet intervention, except where noted below. At 0930 hrs each day, rats were provided with a plastic bottle containing 95 ml of either 10% w/vol glucose (1.53 kJ/g; D+- glucose; Sigma G8270), 9% w/vol sucrose (1.53 kJ/g; table sugar; Coles, Australia), or water (Control group). A 9% sucrose solution was chosen to account for the lower energy density of glucose [37]. Solutions were prepared fresh each day. A daily volume of 95mL for sucrose and glucose solutions was chosen based on our past experiments in individually housed rats, where average daily consumption was around ~110 ml/rat/day for 10% sugar solutions, with a positively-skewed distribution. Therefore, limiting access to 95 ml/day was intended to equate intakes across groups and to preclude differences in consumption of these two sugars, as observed in some past studies [33]. Fluid intakes were recorded daily for the first week and for one 24-h period per week during Weeks 2-7. Body weights and chow intakes were measured weekly. The cage bedding was inspected carefully for fragments of chow.

2.3. 24-h feeding patterns

On Days 1, 15, and 26 of the diet intervention fluid and chow intakes were measured at 0, 3, 6, 9 and 24h. This was to determine whether the partial compensation by sucrose-fed animals observed in our past studies (e.g. [9]) would (a) be evident within the first 24 h of access to the sugar solutions; (b) change across time; and (c) differ for Sucrose and Glucose groups.

116

117 *2.4. Short-term compensation test (Days 18 and 22)*

118 The procedure was based on that used in our previous study [38] that in turn was modelled on
119 Swithers & Davidson [39]. On Day 18, sugar solutions and chow were removed at 1700 hrs, and
120 rats were given 60% of their usual daily chow intake. At 0900 hrs the next day, half the rats
121 (5/group) received a novel pre-meal and half received nothing. The pre-meal was 8g Vanilla
122 Ensure® solution, prepared by dissolving 53.8g of powder in 195ml water. In light of the
123 Glucose and Sucrose groups' extensive prior exposure to sweet solutions, the Ensure solution
124 was thickened with 2% Xanthan gum to provide a novel 'pudding' texture. After 45 min, the pre-
125 meal was removed, chow was replenished, and ~60g of a sweet biscuit (*Nice*^{TM}, 19.1 kJ/g,
126 Arnotts, Australia) was placed in each cage. Chow and biscuit intake were measured following
127 30 min, 2 h, 4 h, and 24 h, at which time sugar solutions were replaced and any biscuit crumbs
128 were removed. After a 3-day washout this compensation test was repeated on Day 22, such that
129 the rats first tested with a pre-meal were now given no pre-meal and *vice-versa*.

130

131 *2.5. Oral glucose tolerance test (Day 28)*

132 On Day 28 of the dietary intervention an oral glucose tolerance test (OGTT) was held after a
133 6-h fast (with water available). After carefully removing the tail tip with a sterile blade, fasting
134 blood glucose was measured using a glucometer (Accu-check® Performa, Roche Diagnostics)
135 and rats were administered a 50% glucose solution by gavage (3 g/kg) with blood glucose
136 measured again after 20, 40, 60, 90 and 120 min. An additional 60 ul blood was collected at
137 fasting for measurement of plasma insulin. The QUICKI index was calculated as a measure of
138 insulin sensitivity ($QUICKI = 1 / [\log_{10}(mg/dl\ gluc) + \log_{10}(uU/ml\ ins)]$) [40].

139

140 *2.6. Object/place task (Days 34-42)*

141 The object and place tasks measure short-term recognition memory by exploiting rats'
142 tendency to approach novel objects or objects in a novel location. In the 5-min *familiarisation*
143 *phase*, the rat is exposed to two identical objects in an otherwise bare arena. In the second
144 *retention phase* the rat is confined to its home cage for 5-min. In the final 3-min *test phase* the rat
145 is returned to the arena, where one of the objects is novel (object task) or has been moved to a
146 new location (place task). The key outcome is the proportion of exploration time directed toward
147 the novel or newly-located object, or Recognition Index: [novel object exploration] / [familiar +
148 novel exploration], where values above 0.5 suggest recognition that something has changed since
149 the familiarisation phase. Chronic access to diets high in sugar and/or fat selectively impairs
150 performance on the place task [30, 41, 42].

151 Testing occurred in a dedicated room separate to where rats were housed. The square test
152 arena had a black PVC floor measuring 60 cm x 60 cm, with 60 cm high walls, and was painted
153 black. A variety of objects of similar size were used (glass bottles, plastic Tupperware
154 containers, tin cans) and were allocated to serve as familiar or novel objects within each group.
155 Rats were first habituated to the empty arena for 15 min on three consecutive days. Testing
156 commenced two days later and was conducted over 4 days. Half of each group were tested each
157 day (n =5/group; N = 15 total), with test sessions starting at the beginning of the dark cycle.
158 Whether rats underwent the place or object test was counterbalanced within each group. A single
159 day separated the two tests for any given rat. The arena and test objects were cleaned with 50%
160 ethanol after each phase. A camera positioned directly above the arena recorded behaviour,
161 which was later scored by a trained observer unaware of experimental group using ODLog ®

software. Exploration was defined as active investigation of the object; proximity to the objects or climbing on top of the objects was not considered exploration. Place test data were excluded for one rat in the Glucose group that failed to explore both objects during the test phase.

2.7. Endpoint (Day 50)

On day 50, rats were euthanased via intraperitoneal injection of sodium pentobarbitone (Lethabarb, 1ml/kg). Retroperitoneal, visceral and epididymal fat deposits were excised and weighed. Experimenters were blind with respect to diet treatment.

2.8. Statistical analyses

All figures show group means ± standard error of mean (SEM). Data were analysed using two planned orthogonal contrasts. *Contrast 1* compared the Glucose and Sucrose groups with the Control group (coefficients: Glucose 1, Sucrose 1, Control -2), whereas *Contrast 2* compared the Sucrose and Glucose groups (coefficients: Glucose 1, Sucrose -1, Control 0). These contrasts were applied to percentage weight gain, average chow consumption (g/day) and total energy intake (kJ/day), fasting glucose and insulin, OGTT incremental area under the curve [AUC], QUICKI index), g/kg fat, and place/object recognition indices. Mixed-ANOVAs (group x time) were applied to absolute body weight gain, short-term compensation tests and consumption of sugar solutions by Sucrose and Glucose groups.

3. Results

3.1. Body weight

Body weight gain across the diet intervention is shown in Figure 1, with percent body weight gain (terminal weight / starting weight * 100 -100) shown inset. Body weight increased significantly over time (linear trend: $F(1, 27) = 427.17$, $p < .001$) but the rate of increase did not differ between groups ($F < 1$). Percent weight gain did not differ between the sugar groups and the Control group (Contrast 1: $F < 1$) nor between the Glucose and Sucrose group (Contrast 2: $F < 1$).

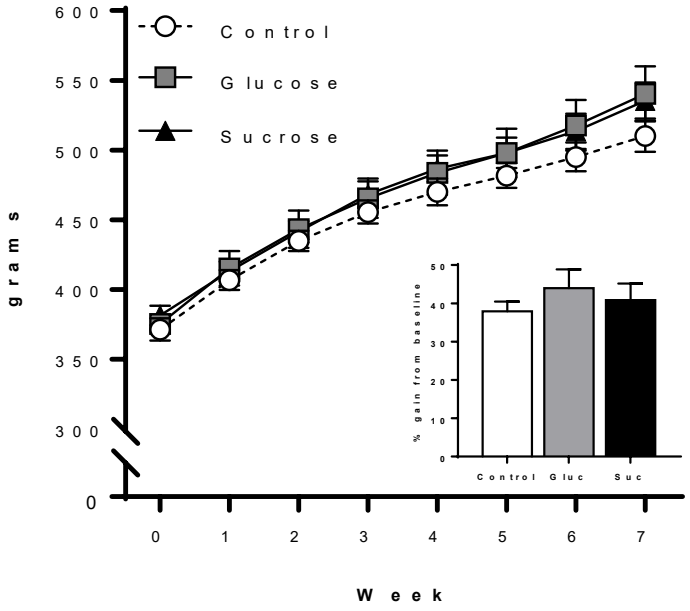

Figure 1. Body weight gain over the 7-week diet intervention. No significant group differences in weight gain were found, nor in percent weight gain (inset).

3.2. Consumption data

Figure 2 displays mean fluid intake (ml/day, Fig. 2a), chow intake (g/day, Fig. 2b) and energy intake (kJ/day; Fig 2b). The Sucrose and Glucose groups did not differ significantly in intake of sugar solutions ($F(1, 18) = 2.63$, $p = .12$). Relative to the Control group, the two sugar groups drank significantly less water (Contrast 1: $F(1, 27) = 312.42$, $p < .001$) and ate significantly less

chow (Contrast 1: $F(1, 27) = 44.21$, $p < .001$). The Sucrose and Glucose groups did not differ in terms of water (Contrast 2: $F(1, 27) = 1.83$, $p = .187$) or chow intake (Contrast 2: $F < 1$), suggesting comparable compensation for the calories from sugar solutions. Finally, sugar-fed groups consumed significantly more total energy than the Control group (Contrast 1: $F(1, 27) = 13.25$, $p = .001$) with no statistically significant difference between the Glucose and Sucrose groups (Contrast 2: $F < 1$). Therefore, sugar-fed groups partially compensated for the calories contained in their sugar solutions. The extent of compensation did not differ significantly according to the nature of the sugar solution provided.

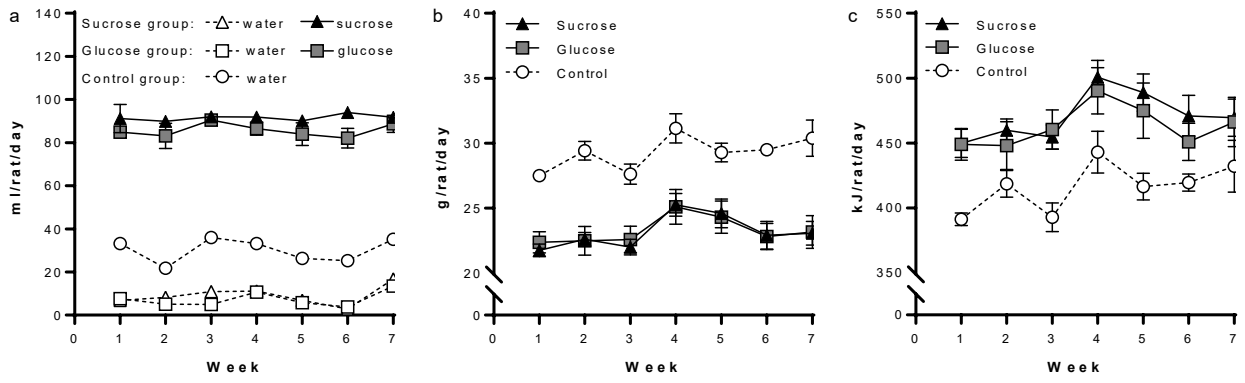

Figure 2. Consumption data. Sucrose and Glucose groups consumed most of their fluid intake as sugar solution (Fig. 2a) and, relative to the Control group, ate significantly less chow (Fig. 2b), but exhibited higher total energy intake (Fig. 2c).

3.3. 24-h feeding patterns

Cumulative energy intake after 3, 6, 9 and 24-h was measured on day 1, 15 and 26 of the diet intervention, with data shown in Figures 3a, 3b and 3c, respectively. On day 1, energy intake by sugar-fed groups did not differ from the Control group after 3 h (Contrast 1: $F(1, 27) = 1.95$, $p = .17$) but was significantly higher after 6 h ($F(1, 27) = 4.68$, $p = .04$), 9-h ($F(1, 27) = 8.50$, $p =$

.007) and 24 h ($F(1, 27) = 8.73$, $p = .006$). Energy intake by Sucrose and Glucose groups did not differ at any time point (Contrast 2: all $F < 1$).

Similar results were observed on day 15 (see Figure 3B), where sugar-fed groups exhibited significantly higher total energy intake after 6 and 9 hours, with non-significant trends in the same direction after 3 and 24 hours (Contrast 1 at 3-h: $F(1, 27) = 3.49$, $p = .073$; 6-h: $F(1, 27) = 7.90$, $p = .009$; 9-h: $F(1, 27) = 13.38$, $p = .001$; 24-h: $F(1, 27) = 2.92$, $p = .099$). Energy intake was significantly higher in the Sucrose group than the Glucose group after 6 and 9-hours, but not statistically significantly different after 3 or 24-h (Contrast 2 at 3-h and 24-h: $F < 1$; 6-h: $F(1, 27) = 4.94$, $p = .035$; 9-h: $F(1, 27) = 5.16$, $p = .031$).

On day 26 of the diet intervention (Figure 3C), sugar-fed groups exhibited higher energy intakes after 6, 9, and 24 but not after 3-h (Contrast 1 at 3-h: $F(1, 27) = 1.32$, $p = .26$; 6-h: $F(1, 27) = 7.36$, $p = .011$; 9-h: $F(1, 27) = 10.51$, $p = .003$; 24-h: $F(1, 27) = 6.13$, $p = .02$). The Sucrose and Glucose groups did not differ significantly at any point (Contrast 2: 3-h and 24-h: $F < 1$; 6-h: $F(1, 27) = 1.49$, $p = .23$; 9-h: $F(1, 27) = 3.09$, $p = .09$).

Finally, the percent of total energy derived from sugar, averaged over the three tests, was 28.73% ± 1.36 [SEM] for the Glucose group and 30.81% ± 1.01 [SEM] for the Sucrose group. These values were not significantly different ($F(1, 18) = 1.51$, $p = .24$) and were highly consistent, with no significant changes across the 3 feeding tests (all $F < 1$).

In summary, these tests revealed that six hours after the introduction of the sugar solutions for the sugar groups, energy intakes were higher in these two groups than in the controls; on Day 15 energy intake by the Sucrose group increased more rapidly than in the Glucose group – as is consistent with the greater palatability of sucrose [31] – but otherwise no differences between these two groups were detected.

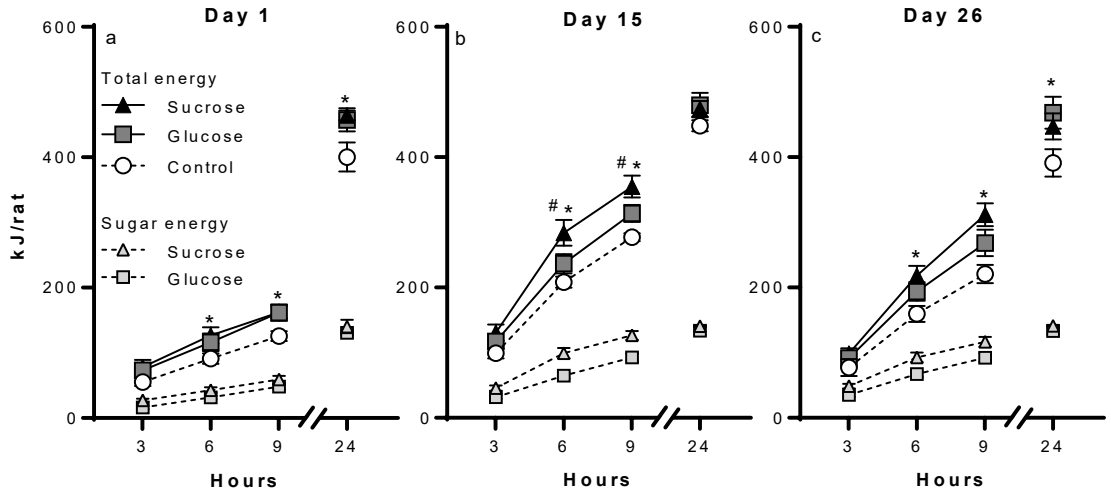

Figure 3. Chow and fluid intakes were measured at 3, 6, 9, and 24-h on day 1 (Fig. 3a), 15 (Fig. 3b) and 26 (Fig. 3c) of the diet intervention. Sugar-fed groups consumed significantly more total energy within the first 9-hours of exposure to solutions. *$p < .05$ for Control vs. sugar-fed groups; #$p < .05$ for Sucrose vs. Glucose group.

3.4. Short-term compensation test (days 18 and 22)

Cumulative energy intake in the compensation tests is displayed in Figure 4. One rat from the Control group and two from the Sucrose group consumed less than 0.3g of the pre-meal and were excluded from analyses. There were no significant differences in pre-meal consumption between groups in the remaining rats (one-way ANOVA; $F < 1$) and analyses of energy intake did not include the calories from the pre-meal. Rats derived most energy from the sweet biscuit on both pre-meal and no pre-meal tests (group means: 61.9 – 75.1%), but the percentage of energy from biscuits did not differ systematically between groups ($F < 1$). There was a trend for rats to consume a higher percentage of energy intake from biscuits on the pre-meal test ($F(1, 24) = 3.57$, $p = .07$).

We first examined effects of the pre-meal during the first 4 hours of the test (see [38]) by analysing non-cumulative energy intakes after 30-min, 2-h and 4-h. Sucrose and Glucose groups were collapsed into a single factor of 'Sugar' and compared with the Control group in a 2 [group: Sugar vs. Control] x (2) [pre-meal: pre-meal vs. no pre-meal] by (3) [time: 0-30min, 30min to 2-h, 2-h to 4-h] mixed-ANOVA. There was a significant main effect of the pre-meal ($F(1, 25) = 4.85$, $p = .037$), qualified by a significant pre-meal x group interaction ($F(1, 25) = 5.67$, $p = .025$), suggesting that compensation differed between sugar-fed and control groups. There were also significant main effects of 'time' ($F(1, 25) = 11.24$, $p = .003$), significant time x group and pre-meal x time interactions ($F(1, 25) = 7.59$, $p = .011$ and $F(1, 25) = 5.20$, $p = .031$), but the group main effect and 3-way interaction were not significant ($F(1, 25) = 3.30$, $p = .081$; $F < 1$, respectively).

To confirm the nature of the pre-meal x group interaction, separate (2) x (3) ANOVAs evaluated the effect of the pre-meal within each group. These analyses revealed a significant pre-meal main effect in the Control group ($F(1, 8) = 9.96$, $p = .013$), but not for the Glucose or Sucrose groups (both $F < 1$) in the first 4-h of the test. However, there were no significant differences in total energy intake between pre-meal and no-pre-meal tests after 24-h ($F(1, 24) = 1.06$, $p = .314$), with no test by group interaction ($F(2, 24) = 1.55$, $p = .23$) and no main effect of group ($F < 1$).

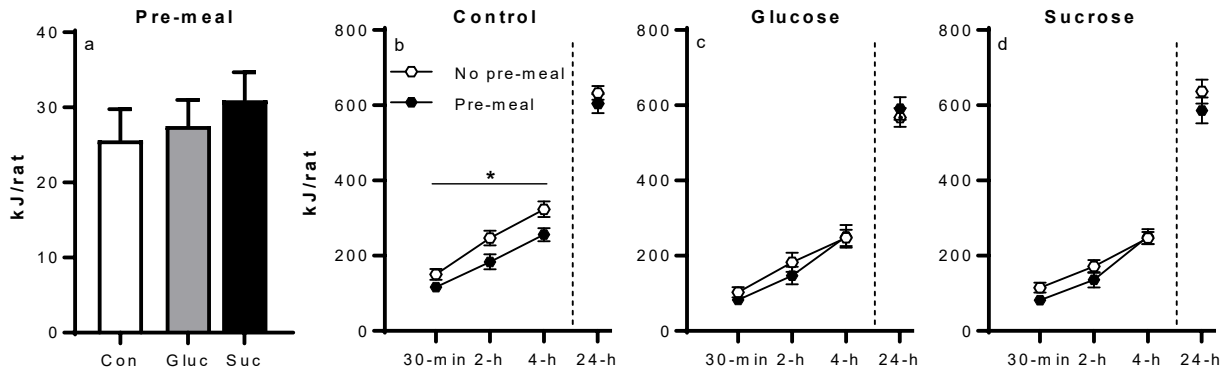

Figure 4. Energy intake in short-term compensation tests. Consumption of the novel pre-meal did not differ between groups (4a). Unlike the Control group (4b), Glucose (4c) and Sucrose (4d) groups did not reduce energy intake during the first 4-h after consumption of a novel pre-meal. Sucrose and glucose solutions were not available during these tests. No effects of the pre-meal were observed after 24-h. *$p < .05$ for 'pre-meal' effect across first 4-hours.

3.5. Glucose tolerance

OGTT data are displayed in Figure 5a and 5b. Two rats (1 Sucrose, 1 Control) did not receive the full gavage load and were excluded. There was no effect of sugar exposure on fasting blood glucose (Contrast 1: $F < 1$) and no significant difference between the Sucrose and Glucose groups (Contrast 2: $F < 1$). Similarly, there were no group differences in the incremental AUC (Figure 5b; both $F < 1$).

3.6. Insulin

Plasma insulin content (pM) and insulin sensitivity (QUICKI index) are displayed in Figure 5c and 5d, respectively. One sample from the Sucrose group haemolysed and could not be analysed. Relative to the Control group, sugar-fed groups exhibited significantly higher fasting plasma insulin (Figure 5c; Contrast 1: $F(1, 26) = 6.82$, $p = .015$) and poorer insulin sensitivity

(Figure 5d; Contrast 1: $F(1, 26) = 7.69$, $p = .01$). Sucrose and Glucose groups did not differ significantly from each other on either measure (both $F < 1$).

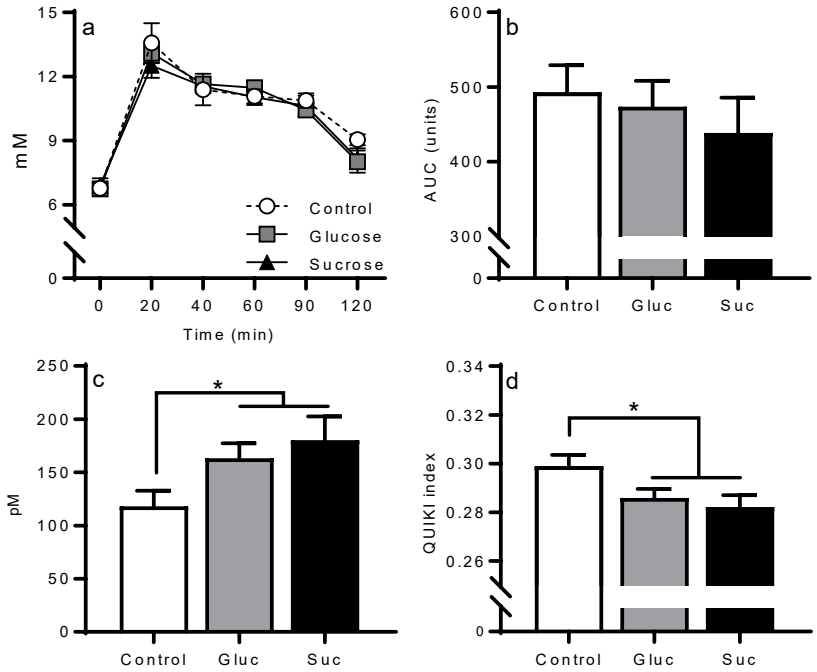

Figure 5. OGTT results. There were no effects of glucose or sucrose consumption on fasting glucose or glucose tolerance (Fig. 5a, 5b); however, sugar-fed groups displayed higher levels of fasting insulin (Fig. 5c) and poorer insulin sensitivity (Fig. 5d) relative to controls. *$p < .05$ for Control vs. Sucrose + Glucose groups.

3.7. Object/Place tasks

Performance in the object and place short-term memory tests is displayed in Figure 6. In the object task there were no effects of sugar and no difference between Sucrose and Glucose groups (Fig. 6c; Contrast 1: $F(1, 27) = 2.26$, $p = .14$; Contrast 2: $F < 1$). On the place task there was no significant difference between the sugar-fed groups and the Control group (Fig 6f; Contrast 1: F

< 1) though there was a non-significant trend toward higher place recognition in the Glucose group than in the Sucrose group (Contrast 2: $F(1, 26) = 3.37$, $p = .078$). Total exploration time did not differ significantly between groups on the object test (Figure 6b; $F(2, 27) = 1.79$, $p = .19$) or place task (Figure 6e; $F < 1$). Similarly, there were no differences in total exploration time during the familiarisation phase in the object test (Figure 6a: $F(2, 27) = 1.34$, $p = .28$) or the place test (Figure 6d, $F < 1$).

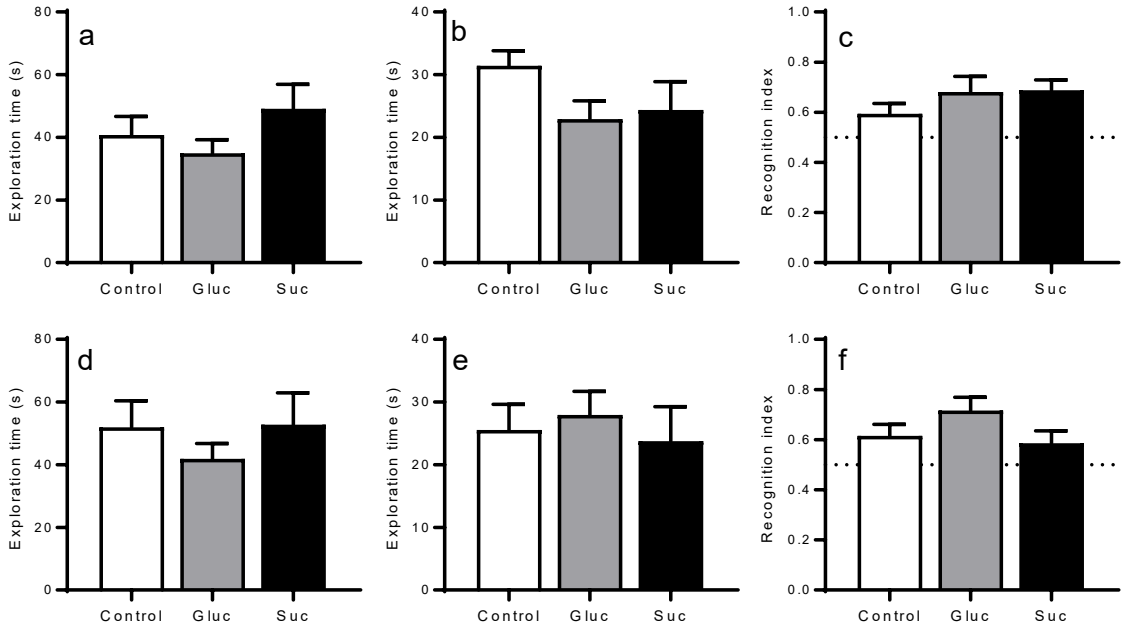

Figure 6. Short-term place and object recognition memory tests. Total exploration of the objects did not differ between groups for the object test (Fig. 6a and 6b for familiarisation and test phases, respectively) or place test (Fig. 6d and 6e for familiarisation and test phases, respectively. Similarly, no significant group differences were found in object recognition (Fig. 6c) or place recognition (Fig. 6f). The dashed line at 0.5 in Figures 6c and 6f indicates equivalent exploration of both objects (i.e. impaired recognition memory).

3.8. Fat mass

Retroperitoneal, visceral and epididymal fat mass as a proportion of body weight (g/kg) are displayed in Figure 7. Relative to the Control group, g/kg retroperitoneal fat (Fig. 7a; Contrast 1: $F(1, 27) = 7.26$, $p = .012$) and epididymal fat (Fig. 7b, $F(1, 27) = 6.98$, $p = .014$) were significantly greater in sugar-fed groups, with a non-significant trend observed for visceral fat (Fig. 7c; $F(1, 27) = 2.40$, $p = .075$). There were no significant differences between the Sucrose and Glucose group at any site (Contrast 2: all Fs < 1).

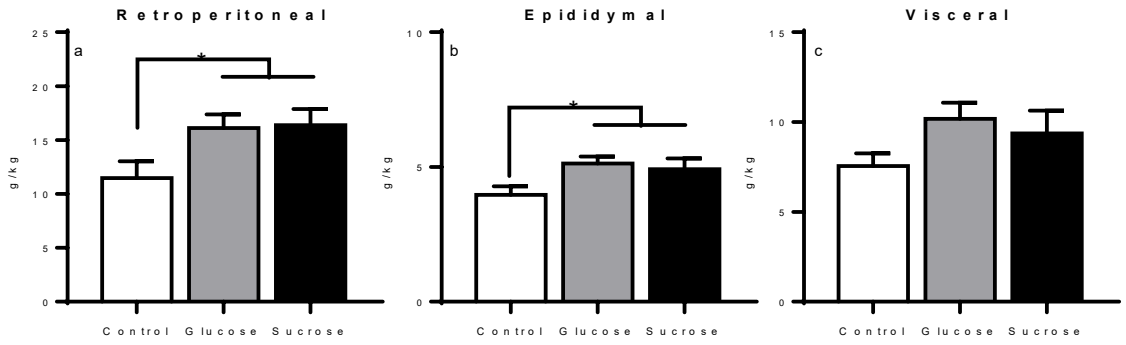

Figure 7. Fat mass at endpoint, expressed as a proportion of body weight (g/kg). Sugar-fed groups had greater retroperitoneal (Fig. 7a) and epididymal fat mass (Fig. 7b) relative to controls, with a non-significant trend observed for visceral fat (Fig. 7c). *$p < .05$ for Control vs. Sucrose + Glucose groups.

4. Discussion

In light of hypotheses that the adverse effects of sucrose are attributable to its fructose moiety, this study compared the effects of limited access (95 ml/day) to isocaloric sucrose and glucose solutions on food intake, compensation for novel sweet foods, glucose tolerance, adiposity and cognitive function. Previous studies comparing sucrose with fructose-free alternatives have

generally allowed *ad-libitum* access, sometimes resulting in group differences in consumption that complicate the interpretation of differences in solid food intake and/or metabolic data. In the present study, capping daily access resulted in comparable daily intakes by Sucrose and Glucose groups, with the two sugars producing similar effects on most parameters.

Previous studies in rats have reported comparable compensation for sucrose and glucose both when administered as an acute pre-load [43-45] and in long-term studies of home-cage chow consumption [31, 33, 34, 46]. Our unpublished pilot data using 10% sucrose and glucose solutions suggested better compensation in rats exposed to glucose than sucrose solutions. However, our hypothesis that the Glucose group would compensate more accurately for the calories in sugar solution via a greater suppression in chow intake was not supported. Relative to controls, Sucrose and Glucose groups reduced chow intake by ~20%, yet this was still less than the calories derived from sugar solutions, serving to increase total energy intake in sugar-fed groups by approximately 12%. Timed analyses of cumulative food intake interspersed throughout the diet intervention indicated that the sugar solutions led to a steady increase in energy intake over the day, which was statistically significant within 6-hours of exposure.

Glucose and Sucrose groups also failed to reduce energy intake when challenged with a novel sweet pre-meal in short-term compensation tests where sweet foods were provided in addition to chow. Thus, while the Control group significantly reduced chow and biscuit intake in the first 4-h after consuming the sweet pre-meal, energy intake by the sugar-fed groups did not differ across two tests, with no differences between Glucose and Sucrose groups. However, these effects were transient, and no group demonstrated sensitivity over 24-h. It should also be noted that the provision of the sweet biscuit increased energy intake for all groups relative to the measures where only chow was available (cf. Figures 3 and 4). Despite the two sugar groups' prior

366 exposure to sweet solutions, all groups consumed a comparable proportion of energy from
367 biscuits versus chow, suggesting that differences in compensation did not result from suppressed
368 consumption of the sweet biscuit in sugar-fed groups due to negative contrast. Instead, group
369 differences were driven by greater intake by the Control group in the no-pre-meal test. An
370 intriguing possibility is that our decision to remove access to sugar solutions for these tests
371 contributed to the suppressed intake by sugar-fed groups in the no-pre-meal test. Comparing the
372 effects of the presence versus absence of sweet solutions during compensation tests will be an
373 interesting future direction. Nonetheless, taken together, these results suggest that a history of
374 sugar intake led to an inability to adjust short-term food intake in response to novel foods. As
375 these compensation tests were implemented relatively early in the present study, it will be
376 informative to examine this form of short-term compensation after longer diet exposures and/or
377 with more pronounced weight gain.

378 Limited daily access to sucrose or glucose did not significantly alter body weight gain over
379 the time course of this study. Although a longer diet exposure may have yielded an effect of
380 sugar exposure on weight gain, it appears unlikely that this would reveal a difference between
381 the Glucose and Sucrose groups, based on the similarity in their energy intake and body weight
382 data. Nonetheless, sugar-fed groups exhibited higher fasting insulin, elevated fat mass, and lower
383 scores on the QUICKI index of insulin sensitivity. The latter result was driven by
384 hyperinsulinemia, as groups did not differ at any point during the oral glucose tolerance test.
385 Altered insulin function in the absence of frank glucose intolerance has been reported in clinical
386 studies [47, 48] with suggestions that hyperinsulinemia precedes the onset of insulin resistance
387 and impaired glucose tolerance [49, 50].

388 Together, our results suggest that restricting access at 95ml/day led to sub-threshold effects on
389 metabolic health. The significant increase in adiposity in sugar-fed groups in the absence of total
390 body weight changes supports this interpretation. Further, in previous studies we have observed
391 increased adiposity, impaired glucose tolerance and elevated fasting glucose following
392 unrestricted access to 10% sucrose solution for at least four weeks in older (4-5 months of age)
393 males [12, 51] and in females [52]. Restricting access also served to reduce the percent of energy
394 from sugar (~31% and ~29% from Sucrose and Glucose groups, respectively) below the ~40%
395 observed in our previous studies, and the ~60% reported in earlier studies, albeit with more
396 concentrated solutions than used here [34]. As these values still exceed estimates of added sugar
397 intake in adults [53], an important future direction will be to study the metabolic effects of sugar
398 drink supplementation at lower proportional levels of intake.
399 No effect of sucrose was observed in the hippocampal-dependent place recognition test,
400 which is often impaired following consumption of diets high in fat and sugar [41, 42, 54, 55] and
401 sucrose or maltodextrin solutions [30]. In our previous sucrose/maltodextrin study, rats given
402 10% sucrose solution obtained around 39% of their energy from this solution [30], again raising
403 the possibility that cognitive impairment is observed only when sugar intake exceeds some
404 proportion of energy intake. There was an intriguing trend toward better place recognition
405 memory in the Glucose group than in the Sucrose group. While this warrants further
406 investigation, as performance was unexpectedly low in the Control group, there is evidence for
407 acute improvements in cognition following glucose ingestion in rodent studies [56]. A recent
408 systematic review and meta-analysis of human experimental studies found modest evidence that
409 glucose improved verbal memory recall, but reported a high risk of bias, and called for further
410 work on sucrose [57].

411 This work extends previous studies that have compared the metabolic effects of sucrose and
412 glucose in rats using highly concentrated solutions [33], or over short periods of time without
413 consideration of chow consumption [35]. The present results suggest that the difference in
414 weight gain found by Kanarek & Orthen-Gambill [33] may not have emerged if access to
415 solutions was capped as in the present study. Whether they still would have found greater
416 glucose intolerance in the sucrose than glucose group remains an open question.
417 In summary, the present results are consistent with past reports [34-36], in that few
418 differences between Sucrose and Glucose groups was detected in terms of metabolic measures.
419 This corroborates the results of our previous comparison of sucrose with maltodextrin [30] and
420 suggests that when ecologically valid concentrations (i.e., ~10%) of sugar solutions are provided
421 to rats, the fructose-containing disaccharide sucrose produces similar metabolic damage to
422 fructose-free mono- or oligosaccharides such as glucose or maltodextrin.
423

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