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ORIGINAL CONTRIBUTION

Metabolic and behavioural effects of sucrose and fructose/glucose drinks in the rat

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Abstract
Purpose Overconsumption of sugar-sweetened beverages, in particular carbonated soft drinks, promotes the development of overweight and obesity and is associated with metabolic disturbances, including intrahepatic fat accumulation and metabolic syndrome. One theory proposes that drinks sweetened with high-fructose corn syrup are particularly detrimental to health, as they contain fructose in its 'free' monosaccharide form. This experiment tested whether consuming 'free' fructose had a greater impact on body weight and metabolic abnormalities than when consumed 'bound' within the disaccharide sucrose.
Methods Male Hooded Wistar rats were given free access for 56 days to 10% sucrose (Group Suc), 10%, 50/50 fructose/glucose (Group FrucGluc) or water control drinks (Group Control), plus chow. Caloric intake and body weights were measured throughout the protocol, and the following metabolic indices were determined between days 54 and 56: serum triglycerides, liver triglycerides, retroperitoneal fat and oral glucose tolerance.
Results Animals with access to sugar beverages consumed 20% more calories, but did not show greater weight gain than controls. Nevertheless, they developed larger abdominal fat pads, higher triglyceride levels and exhibited impaired insulin/glucose homeostasis. Comparison of the two sugars revealed increased fasting glycaemia in the FrucGluc group, but not in Suc group, whereas the Suc group was more active in an open field.
Conclusions A metabolic profile indicating increased risk of diabetes mellitus and cardiovascular disease was observed in animals given access to sugar-sweetened beverages. Notably, 'free' fructose disrupted glucose homeostasis more than did 'bound' fructose, thus posing a greater risk of progression to type 2 diabetes.

Keywords Metabolic syndrome · Sucrose · Fructose · Glucose · High-fructose corn syrup · Insulin resistance · Glucose tolerance · Triglycerides · Behaviour

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Introduction

Sugar-sweetened drinks play an important role in the worldwide increase in the prevalence of overweight and obesity. This has been suggested in view of strong parallel trends between rising intakes of sugar-sweetened beverages and long-term weight increases at the population level [1]. The majority of current meta-analyses and reviews support the notion that overconsumption of sugar-sweetened beverages, in particular carbonated soft drinks, promotes the development of overweight and obesity [2–4]. In addition, sugar-sweetened beverages have been associated with a range of other metabolic disorders including metabolic syndrome [5, 6], type 2 diabetes [7, 8], non-alcoholic fatty liver (NAFLD) [9, 10] and coronary heart disease [11].

Sugar-sweetened beverages generally contain one of two sugars. In the USA, today the most common sweetener is high-fructose corn syrup (HFCS55)—a mixture of 55% fructose and 42% glucose monosaccharides, together with 3% glucose polysaccharides. In most of the rest of the world, sucrose, a disaccharide composed of glucose and fructose, continues to be used as the primary sweetener [12].

It has been suggested that fructose, as opposed to glucose, is the critical component that induces weight gain and metabolic derangements associated with sweetened beverage consumption [1, 13, 14]. Ingestion of fructose does not stimulate release of the anorexigenic hormones insulin and leptin and does not suppress secretion of the orexigenic hormone ghrelin, to the same extent as other dietary components such as glucose (reviewed in [13]). Attenuation of satiety hormone release can lead to overeating which, in the absence of compensatory measures such as increased activity, promotes body weight gain. An additional compounding effect of sweetened drinks pertains to their liquid form with prior research demonstrating calories consumed as liquids are less satiating than those consumed as solids; hence, consuming calories in beverage form further stimulates caloric overconsumption [15–17].

Excessive fructose intake has additional metabolic repercussions. Fructose is primarily metabolized in the liver (50–75%) where it is metabolized to form lipogenic substrates, such as glyceraldehyde 3-P, acetyl CoA and diacylglycerol, which promote lipid synthesis. Enhanced lipid synthesis contributes to dyslipidaemia, ectopic lipid deposition in liver and muscle tissue, and promotes hepatic and whole body insulin resistance. Comparatively, glucose is far less lipogenic than fructose; glucose metabolism is ubiquitous throughout the body, and its rate of entry into the glycolytic pathway when it does reach the liver is tightly regulated by the action of insulin and negative feedback mechanisms in response to cellular energy status [14].

As the two commonly used sweeteners, HFCS 55 and sucrose, contain the same constituent sugars, glucose and fructose, in roughly equal proportions, the negative health effects of HFCS and sucrose are generally considered quantitatively similar [12]. In contrast, Bray et al. [18] suggested that HFCS was a major contributor to the rising rates of obesity in the USA, arguing that the shift starting in the late 1960s from using sucrose as a primary sweetener to HFCS had led to a major increase in 'free' fructose consumption and this had been particularly detrimental to human health. However, only a few studies to date have directly compared the physiological effects of 'free' against 'bound' fructose. A number of recent review articles have noted this as a critical research gap within the area [12–14].

The present study was prompted by two experiments reported by Bocarsly et al. [19]. In their first experiment, male rats given 12-h access to HFCS55 solution for 8 weeks gained more weight than rats given 12-h access to sucrose solution. In their second experiment, female rats were given 12- or 24-h access to HFCS, sucrose or control beverages for a period of 7 months. The rats given 24-h access to HFCS gained more weight than both the rats given 12-h access to sucrose drinks and the controls. The HFCS group also had elevated non-fasting serum triglyceride levels compared to animals given sucrose, and a trend towards higher uteral, abdominal and intestinal fat pads was observed. These outcomes make a prima facie argument that drinks sweetened with 'free' fructose in HFCS are more harmful in terms of body weight and metabolic measures than sucrose-sweetened beverages.

A second study directly comparing the chronic effects of free and of bound fructose focused on the liver [20]. Rats given isocaloric feeds comprising 30% caloric intake from glucose and 30% from fructose (60% in total) for 16 weeks had significantly higher hepatic fatty acid deposits on histological analysis and tended to have higher liver triglyceride levels when compared to animals receiving a diet comprised of 60% sucrose. In this study, however, neither sugar produced an increase in body weight or central adiposity.

The present study aimed (1) to determine whether chronic sweetened-drink consumption negatively impacts on body weight and metabolic parameters, and (2) to compare the effects of sucrose with those of its constituent monosaccharides, fructose and glucose. Based on the literature presented above, it was hypothesized that the two groups given sugar-sweetened beverages, taken together, would demonstrate greater body weight gain, adiposity and metabolic disturbances compared to water control animals. Furthermore, we predicted a significant divergence in terms of body weight gain and metabolic outcomes between the two sugar-sweetened beverage groups, namely fructose/glucose and sucrose.

Methods

Animals

Thirty male Hooded Wistar rats (*Rattus norvegicus*) were initially obtained from Laboratory Animal Services, Adelaide University and were allocated in equal numbers to the three groups by matching for body weights. They were approximately 125 days old at the start of this experiment after previously serving in a behavioural experiment in which tone and light cues signalled the delivery of food pellets. The animals were individually housed in plastic

tubs (46 × 27 × 32 cm) in a 12:12 h light–dark cycle (lights on at 07:00 h), temperature (22–24 °C) and humidity-controlled (40–50%) holding room.

All procedures were approved by the University of Sydney Animal Ethics Committee.

Dietary intervention procedure

Over a 56-day period, all animals were given unrestricted access to chow (Specialty Feeds®, 3.4 kcal/g; 20% protein, 4% fat, 60% carbohydrate), water and the following drinks: Group Suc, 10% sucrose solution (CSR® white sugar, w/v made up on tap water; 0.4 kcal/mL); Group FrucGluc, a 10% solution (w/v made up on tap water; 0.4 kcal/mL) containing 50/50 (mol/mol) fructose (SF Health Foods®) and glucose (Univar® anhydrous D-Glucose); while Group Control was given water as the sole beverage. The choice of 10% sugar solutions was based on the caloric density of popular carbonated beverages (~0.4 kcal/mL).

Body weight and intake measures

Animals were weighed every 6 days for a total of 54 (of 56) days on the dietary regimen. Drink bottles and residual chow were weighed and replenished every 3 days at approximately the same time of day (0900–1030 h). To optimize the accuracy of chow intake calculations, chow segments distributed throughout the bedding were collected and weighed with the residual chow. Bedding was changed every 3 days immediately after the drinks were weighed. To assess the volume losses that occurred as a result of bottle leakage, 'control' bottles containing either sucrose or fructose/glucose solutions were set up and handled identically to those used in the experimental protocol, but in the absence of a test animal. Drink bottle spouts were rotated among the test animals and control bottles when drinks were replenished. Calculations of daily caloric intake from drinks included a correction for losses (mean 1.05%) in control bottles.

Insulin sensitivity and glucose homeostasis measures

On the morning of day 54, all food and sweetened drinks were removed from the cages and the animals were fasted for 6 h. Fasting glucose was measured using a diagnostic glucometer (Accu-chek® Performa, Roche Diagnostics) applied to a cut in the tail vein of each animal. Fasting insulin levels were later assessed by using insulin (rat) ELISA kit (ALPCO®, cat. # 80-INSRT-E01). The quantitative insulin-sensitivity check index (QUICKI), a surrogate measure of insulin resistance, was calculated as follows: $\text{QUICKI} = 1/[\log(I_0) + \log(G_0)]$, where I is fasting plasma insulin levels (μU/mL) and G is fasting blood glucose (mg/dL). The use of QUICKI has been validated against the hyperinsulinaemic euglycaemic clamp in the rat [21].

An oral glucose tolerance test (OGTT) was performed on 6 rats from each group, matched by mean fasting body weight determined on the day of the OGTT (473 g). A glucose load was administered by oral gavage (3 g/kg body weight), and blood glucose levels (BGL) were measured at 0, 20, 40, 60, 90 and 120 min post-gavage, using a diagnostic glucometer (Accu-chek® Performa). Area under the glucose curve (AUC) during the OGTT was calculated using the trapezoidal method of integration. All animals were subsequently returned to their cages and given their original test diet for a further 2 days.

Fat pads, hepatic triglyceride and blood lipid measures

On day 57, rats were euthanazed by intraperitoneal injection of pentobarbitone sodium anaesthetic euthanasia solution (Lethabarb®; ~60 mg/kg body weight) 2 h after the start of the light-on cycle and sacrificed through cardiac puncture. During cardiac puncture, blood samples were collected from the right atrium, and the serum frozen at −20 °C, for the non-fasting triglyceride (TAG) assay. Retroperitoneal fat pads were collected and weighed. The right upper lobe of the liver was removed, snap-frozen with liquid nitrogen and stored at −80 °C for determining liver TAG content.

Liver tissue was homogenized in 2:1 chloroform/methanol solution. Following 2.5-h incubation at room temperature (20–24 °C), 0.9% NaCl was added in a 6:1(v/v) ratio and the sample centrifuged (2,000 rpm, 30 s). The supernatant was removed and the sample allowed to dry for 36 h. Dried samples were dissolved in 100% ethanol. The TAG content of liver samples and non-fasting serum triglycerides were measured using triglycerides endpoint calorimetric method kit (Thermo Fisher Scientific, West Sussex).

Behavioural test procedures

These tests were conducted following informal observations suggesting unanticipated behavioural differences between the groups. The animals remained on their test diet throughout these tests, except when transferred to behavioural laboratories. All tests were carried out during the light phase, and the groups were counterbalanced with respect to time of testing.

An open field test using a 'locomotor' chamber was run on day 49, with the room, apparatus and recording software set up as previously described in Van Nieuwenhuijzen et al. [22], except in the absence of ambient heating. The same recording apparatus and computer set-up was used in all

subsequent tests. The following behaviours were recorded over a period of 60 min: travelled activity (distance travelled through space) (m), non-travelled activity (centre of mass stationary with body moving) (m), total activity (travelled activity + non-travelled activity) (m) and hide time (time spent in the hide box) (min).

On day 50, animals were first given the emergence test, as described previously [23]. This test measured both anxiety-related behaviour and locomotor activity. The following measures were recorded over 5 min: Total activity, emergence time (time taken for animals to fully exit the hide box), and the time spent on the far side.

On completion of the emergence test, each animal was placed in the elevated plus maze (EPM) to assess anxiety-related behaviour. The testing was carried out as previously described [24] and the following behaviours scored over a period of 5 min: Per cent open time (percentage of time on the open arms as a function of total arm time); time in the central square; and time spent in risk assessment (centre of mass in the closed arms with the head protruding into an open arm).

Data analysis

Statistical analysis was performed using SPSS statistical software for Windows (version 19.0; SPSS Inc, Chicaco, IL). The criterion for significance was set at $p < 0.05$. All metabolic measures, total caloric intake and caloric intake from chow were analysed using one-way analysis of variance (ANOVA), with treatment group as factor. Planned orthogonal contrasts were subsequently employed to compare the effects of (a) sugar drinks versus water control, and (b) sucrose versus fructose/glucose. Trends in body weight over time and blood glucose curves throughout the OGTT were additionally examined using repeated measures ANOVA. As for the behavioural tests, locomotor box data were analysed using ANOVA, and a one-way analysis of covariance (ANCOVA) was used for the emergence test and EPM, with time of day as a covariate. The latter reflected the sensitivity of behavioural measures to time of day. As there was no a priori basis for planned contrasts of behavioural measures, treatment effects were explored by pair-wise comparison testing, with Bonferroni adjustment for multiple comparisons.

For all data, homogeneity of variances was assessed through Levene's test, and Kolmogorov–Smirnov test was used to assess normality of data. Where data did not conform to a normal distribution, log transformations were applied, and the geometric mean and 95% confidence intervals are presented herein. Data for normally distributed variables is presented as mean ± standard deviation (SD).

Results

Exclusion of animals

Three of the thirty rats were excluded from analysis, one from each of the groups. Two suffered self-inflicted injuries and a third rat was excluded because its body weight remained more than two standard deviations below the group mean during the first 6 weeks of the dietary protocol.

Body weight and intake measures: despite increased calorie intake by groups Suc and FrucGluc, these groups gained weight no faster than Group Control

Body weights at baseline, final body weight and body weight gain did not differ between groups, as shown in Table 1. Over days 1–54, there was a linear increase in body weight, $F(1, 24) = 632.2$, $p < 0.001$, with neither a significant main effect of group nor a significant group × trend interaction, both Fs < 1.

Uniform body weight gain across the three groups occurred despite marked differences in total daily energy intake, $F(2, 24) = 19.13$, $p < 0.001$ (see Fig. 1), with rats given the sugar drinks (Suc and FrucGluc) consuming 19.6% greater total daily caloric intake than controls, $t(24) = 5.95$, $p < 0.001$. Energy intake from chow was also significantly different in the three groups: $F(2, 24) = 34.80$, $p < 0.001$; animals given sugar drinks partially

Table 1 Body weight of animals at baseline and at 54 (of 56) days on the dietary regimen; animals were given either sucrose drink (Suc group), fructose and glucose drink (FrucGluc group) or water only (Control group)

Measures	Group			*t* test probability	
	Suc ($n = 9$)	FrucGluc ($n = 9$)	Control ($n = 9$)	Sugar versus control (1-tailed)	Suc versus FrucGluc (2-tailed)
Baseline body weight (g)	387 ± 35	384 ± 12	380 ± 35	>0.10	>0.10
Final body weight (g)	478 ± 34	476 ± 13	466 ± 43	>0.10	>0.10
Body weight gain (g)	91 ± 16	92 ± 15	86 ± 15	>0.10	>0.10

Data are presented as mean ± SD

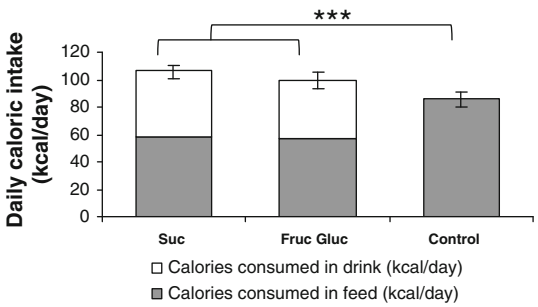

Fig. 1 Daily energy intake from drinks, chow and total caloric intake. Groups consumed either sucrose (Suc group), fructose and glucose (FrucGluc group) or water (Control group). *Error bars* depict 95% CI of total energy intake. *** $p < 0.001$ sugars versus control for total energy intake and energy from chow

compensated for calories consumed in drink, ingesting 33.0% less calories from chow than the water control group, $t(24) = 9.07$, $p < 0.001$.

No significant differences between Group Suc and Group FrucGluc were detected on any of the dietary intake measures (see Fig. 1). Although total daily caloric intake was slightly higher in Group Suc compared to Group FrucGluc, this did not reach statistical significance; $t(24) = 1.69$, $p = 0.10$.

Glucose metabolism: both sugars reduced glucose tolerance and peripheral insulin sensitivity, but only fructose/glucose produced abnormally high fasting blood glucose levels

The animals were adequately fasted prior to the OGTT, insofar as animal mean body weights were lower after the fast (463 ± 7 g) compared to pre-fasting weights (470 ± 7 g), $t(27) = 23.72$, $p = < 0.001$. During the 2-h OGTT, the glucose level across animals changed significantly with time, $F(4, 60) = 2.16$, $p < 0.001$.

As illustrated in Table 2, animals given sugar drinks had significantly higher fasting insulin levels and lower insulin sensitivity, than water controls, $t(24) = 2.97$ and $t(24) = 3.62$, both $ps < 0.05$. Fasting blood glucose levels were also higher in rats given sugar drinks than controls, $t(24) = 2.91$, $p < 0.05$ (see Table 2) with significantly higher fasting glucose levels in the Group FrucGluc compared to Group Suc, $t(24) = 4.53$, $p < 0.001$. Groups Suc and FrucGluc did not differ in fasting insulin or the QUICKI, $t(24) = 1.40$ and $t(24) < 1$, both $ps > 0.10$.

The glucose AUC and the 2-h blood glucose levels were higher in sugar drink groups than the Control group $t(15) = 2.45$ and $t(15) = 2.85$, respectively, both *p-values* < 0.05 (see Fig. 2). The AUC for Groups Suc and FrucGluc were similar, $t(15) = 1.02$, and 2-h BGL, all *p-values* > 0.1.

Tissue analysis and blood lipid levels: both sugars produced high liver and serum triglycerides and larger retroperitoneal fat deposits, and the effects of the two types of sugar were indistinguishable

Data were lost from one liver sample in the FG group as a result of spillage, and consequently, only 8 animals from this group were included in data analysis. As shown in Fig. 3, the sugars had a negative effect on the three metabolic parameters measured. Abdominal adiposity, as indicated by retroperitoneal fat mass, was larger in the sugar groups than in controls, $t(24) = 2.15$, $p = 0.020$. Animals in the sugar groups also displayed higher liver and higher serum triglyceride levels, $t(23) = 3.71$ and $t(23) = 3.64$, respectively, both $ps < 0.01$. Group Suc and Group FrucGluc did not differ in terms of serum or liver triglycerides or in relative retroperitoneal fat pad mass, largest $t(23) = 1.703$, $p > 0.10$.

Behavioural data: Group Suc was more active than the other two groups, but no group difference in anxiety was found

As shown in Table 3A, travelled activity, $F(2,24) = 4.8$, $p = 0.018$, non-travelled activity, $F(2,24) = 6.8$, $p = 0.005$, and total activity, $F(2,24) = 6.3$, $p = 0.006$, differed between groups in the locomotor test. Non-travelled activity and total activity were greater in Group Suc than in Group FrucGluc, $p = 0.008$ and $p = 0.023$ respectively, and Group Control, $p = 0.014$ and $p = 0.009$,

Table 2 Fasting insulin, fasting blood glucose level (BGL) and the quantitative insulin-sensitivity check index (QUICKI) in sucrose (Suc), fructose/glucose (FrucGluc) or water (Control) groups

Measures	Group			*t* test probability	
	Suc ($n = 9$)	FrucGluc ($n = 9$)	Control ($n = 9$)	Sugar versus control (1-tailed)	Suc versus FrucGluc (2-tailed)
Fasting BGL (mmol/L)	6.8 ± 0.3	7.5 ± 0.5	6.7 ± 0.3	0.004	<0.001
Fasting insulin (ng/mL)	2.8 ± 1.0	2.3 ± 0.7	1.6 ± 0.5	0.003	>0.10
QUICKI	0.256 ± 0.010	0.259 ± 0.010	0.273 ± 0.0113	<0.001	>0.10

Data are presented as mean ± SD

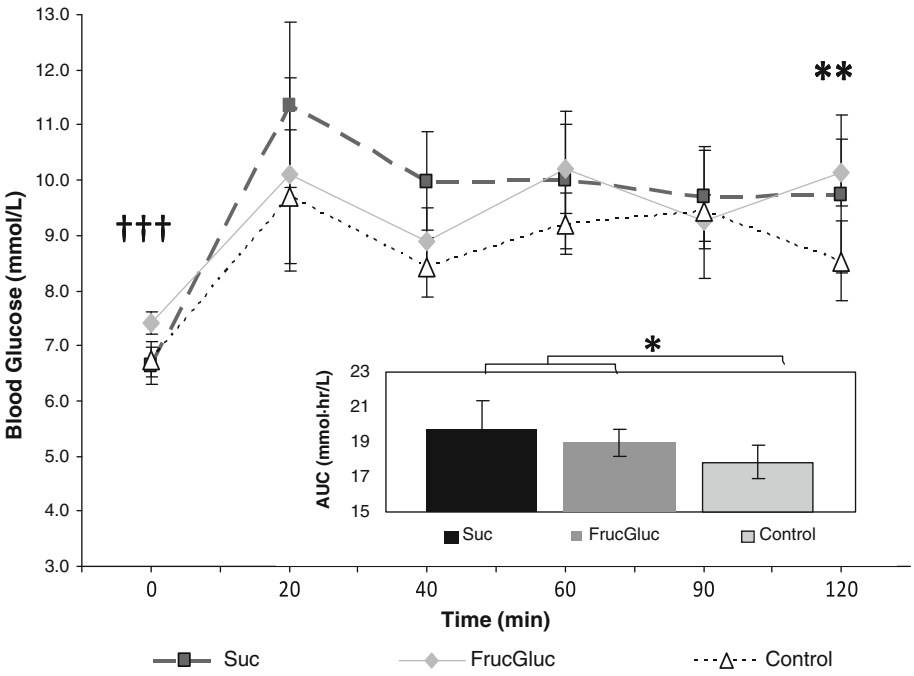

Fig. 2 Blood glucose curves during the 2-h oral glucose tolerance test (OGTT) and the respective area under the 2-h glucose curve (AUC) for each group. Animals were given sucrose (Suc), fructose and glucose (FrucGluc) or water drinks (Control). Six rats were used from each of the original drink groups ($n = 9$) for the OGTT and were selected by matching for mean body weight, prior to the OGTT. Points depict mean ± SD. ** $p < 0.01$, * $p < 0.05$ sugar groups (FrucGluc and Suc) versus Control, ††† $p < 0.001$ Suc versus FrucGluc

respectively. Travelled activity was also significantly greater in Group Suc than in Group Control, $p = 0.019$, but failed to reach significance when Suc was compared to FrucGluc, $p = 0.083$. No differences between Group FrucGluc and Group Control in the locomotor chamber were detected on any of these measures, all ps > 0.10. A similar pattern of results in relation to activity was obtained from the emergence test (Table 3B) but in this case the group differences just failed to reach significance, largest $F(2,23) = 3.4$, $p = 0.05$ for an overall group effect after adjustment for time of day (see Table 2).

As for anxiety measures (Table 3B, C), no differences between the groups were detected in either the emergence test, largest $F(2,23) = 2.4$, $p > 0.10$ (for time spent on the far side) or in the elevated plus maze, largest $F(2,23) = 1.06$, $p > 0.10$ (for time spent in the central square). As anxiety-related behaviour did not differ between the groups, activity measures were not confounded by differences in anxiety.

Discussion

Despite consuming many more calories than the control group, body weight increases in sugar-consuming rats were no greater than in controls. Nevertheless, the sugar groups presented with greater abdominal fat mass, higher levels of serum and liver triglycerides, impaired glucose tolerance and fasting hyperinsulinaemia. Taken together, sugar groups displayed outcomes indicative of progression towards the metabolic syndrome, the components of which constitute an increased risk of developing type 2 diabetes and accompanying cardiovascular and renal disease [24].

The second major finding came from comparing the two sugar groups. The FrucGluc group displayed a more deleterious metabolic outcome than the Suc group in that fasting hyperglycaemia developed only in the FrucGluc group.

Effects of chronic sugar drink consumption on body weight and metabolic measures

Provision of sugar drinks in addition to regular chow is generally accompanied by increased caloric intake in the range of 10–20% [25]. Accordingly, in the present study, the two sugar groups consumed 19.7% more calories on average than controls (see Fig. 1). Although animals given sugar-sweetened beverages partially compensated for calories in drinks through reduced intake of chow (see Fig. 1), the compensation was incomplete, leading to an overall increase in daily caloric load. Incomplete compensation for calories consumed in sugar-sweetened beverages was anticipated from the lesser impact of sugar-sweetened beverage on satiety signals, as outlined in 'Introduction'.

It was interesting to note that this increased caloric intake did not produce greater body weight gain (see Table 1). There has been marked variability in terms of weight gain outcomes in studies investigating the effects of sweetened-drink consumption in rats [25]. The observation that animals given sugar-sweetened drinks consumed an average of 33% less chow than water control animals is of particular importance in the present study. The two sugar

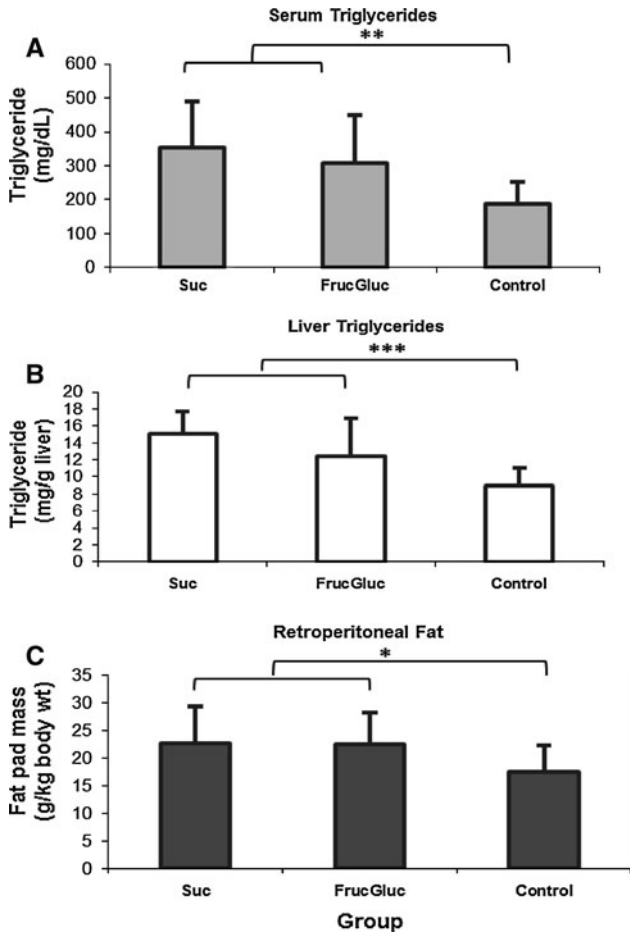

Fig. 3 Effects of sugar drinks on three metabolic measures: **a** non-fasting serum triglycerides; **b** liver triglyceride content; and **c** retroperitoneal fat stores. The rats had received either sucrose (Suc group), fructose and glucose (FrucGluc group) or water (Control group). *Columns* depict mean + SD. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

groups had a higher energy intake, yet their diet was also of lower nutritional quality, incorporating lower protein, micronutrients, essential fatty acids, fibre and trace elements from chow. Such modifications to an animal's diet can result in complex and widespread effects on an animal's body weight and metabolic outcomes. For example, weight-loss is a recognized feature of vitamin A and zinc deficiencies [26, 27]. Moreover, low-protein diets are known to stimulate sympathetic nervous system activity (SNS), promote enhanced metabolic heat production and thereby counteract body weight gain [28]. The choice of an unusual rat strain, the Hooded Wistar, a pigmented animal, in the present experiment likely compounded this effect. The majority of studies investigating effects of supplementary sugar drinks have employed albino rat strains, usually Sprague–Dawley or albino Wistar. Compared to pigmented rats, albino rats have diminished metabolic rate, altered enzyme levels in kidney and liver cells (e.g. cytochrome P-450), as well as possibly attenuated release of CNS neurotransmitters, dopamine, epinephrine and norepinephrine, involved in SNS function [29, 30]. It is therefore reasonable to theorize that pigmented rats, such as the Hooded Wistar, would display greater resistance to body weight gain when consuming a low-protein diet.

Alternatively, the absence of weight gain in the presence of increased energy intake suggests that animals given sweetened drink had a higher energy expenditure than controls. We did attempt to investigate some measures of activity (see below). However, more comprehensive study designs that include whole body calorimetry and/or 24-h habitual activity monitoring would be of interest.

An important point indicated by the present experiment is that weight gain can be a poor indicator of increased

Table 3 Results from three behavioural tests

Behavioural test	Measures	Group		
		Suc ($n = 9$)	FrucGluc ($n = 9$)	Control ($n = 9$)
A. Locomotor box	Travelled activity (m)	$32.7 \pm 9.3^{*}$	23.6 ± 8.7	20.8 ± 7.7
	Non-travelled activity (m)	$22.8^{*\dagger\dagger}$ [19.3–26.6]	$15.6^{\dagger\dagger}$ [13.1–18.4]	15.9 [12.2–20.2]
	Total activity (m)	$56.0 \pm 13.7^{\dagger***}$	$39.4 \pm 9.7^{\dagger}$	37.0 ± 12.5
	Hide time (min)	19.1 [10.2–30.7]	35.12 [24.0–48.3]	33.08 [21.1–47.7]
B. Emergence test	Total activity (m)	25.7 ± 2.0	22.9 ± 4.0	22.9 ± 3.7
	Emergence time (s)	0.8 [0.2–12.0]	41.0 [5.4–276.8]	9.1 [2.8–26.1]
	Time spent on the far side (s)	95.8 ± 31.9	74.0 ± 37.6	65.1 ± 41.4
C. EPM	% open time	15.5 [6.9–27.5]	11.1 [4.2–21.3]	12.1 [4.9–22.7]
	Risk assessment (s)	3.9 ± 2.1	3.3 ± 1.4	3.5 ± 1.9
	Central square (s)	18.0 ± 10.4	29.0 ± 11.6	23.0 ± 16.8

A. Locomotor box, B. Emergence test and C. Elevated plus maze (EPM)

The rats had received either sucrose (Suc group), fructose/glucose (FrucGluc group) or water (Control group)

Values are shown as mean ± SD or, where data were transformed to adhere to normality, the geometric mean and [95% CI] are presented. * $p < 0.05$, ** $p < 0.01$ versus control. †† $p < 0.05$, †† $p < 0.01$ versus other sugar group (Suc or FrucGluc)

adiposity in response to excessive and prolonged intake of sugar beverages. Comprehensive analyses of body composition such as body fat content and fat pad mass are necessary outcome measures in the evaluation of adiposity and metabolic risk. Importantly, the present study has identified elevated abdominal fat pad mass and serum triglycerides to accompany elevated liver triglyceride content and peripheral insulin resistance, supporting a role for sugar drinks in the development of metabolic syndrome and fatty liver disease (See Table 2, Fig. 3).

In the light of the present findings, it may be suggested that the ability of sweetened drinks to induce these cardiometabolic risk factors is a consequence of consuming a diet of poor nutritional quality as opposed to overconsumption of the fructose component of the sugars. However, fructose has been shown to induce symptoms of the metabolic syndrome in animals, and these results have not been observed in controls given an equivalent diet and equal quantities of glucose [31]. Intervention studies have similarly demonstrated that fructose-sweetened drinks can induce these features in humans [32–34]. For example, Stanhope et al. [32] administered fructose or glucose drinks to overweight women at 25% of energy requirements over a period of 10 weeks. They found that the fructose, but not the glucose drink increased visceral adipose volume, reduced insulin sensitivity and produced a more atherogenic lipid profile marked by increased fasting and oxidized LDL cholesterol, fasting apolipoprotein B and increased 24-h triglyceride levels. The glucose drink increased subcutaneous fat only, which is known to be considerably less metabolically active and less harmful to health [35]. The ability of sweetened drinks to induce observed cardiometabolic risk factors in the present study therefore stems, at least in part, from the fructose component of the sugars.

Differences between sucrose and fructose/glucose drinks

Both sugar groups, Suc and FrucGluc, developed impaired glucose tolerance (IGT), which is indicative of peripheral insulin resistance (see Table 2, Fig. 2). On the other hand, only FrucGluc had the additive detriment of impaired fasting glucose (IFG), suggesting this sugar prompted the development of hepatic insulin resistance [36]. In prospective epidemiological studies, the combined effect of hepatic and peripheral insulin resistance was shown to be associated with a twofold greater risk of progression to type 2 diabetes than IGT alone [37]. These findings indicate that FrucGluc drinks promote a more deleterious metabolic profile than those sweetened with Suc and lend further support to the notion that the structural form in which fructose-containing sugars are consumed is physiologically pertinent.

The difference in hepatic insulin resistance observed in the two sugar-consuming groups, however, is unusual in that hepatic insulin resistance has been suggested to be induced by hepatic lipid deposition [14]. In the present study, however, we observed raised liver triglyceride levels in both sugar-receiving groups, while fasting hyperglycaemia was restricted to the FrucGluc group. These findings suggest that hepatic insulin resistance and hepatic lipid deposition arise from distinct processes in this dietary model. Faeh et al. [38] reached a similar conclusion. They overfed fructose to healthy human males for 6 days and found that this increased hepatic de novo lipogenesis, raised serum triglycerides and increased fasting glycaemia. However, while fish oil supplementation, given prior to and throughout fructose overfeeding, reduced triglyceride levels in these participants, it failed to reduce fasting glycaemia or hepatic insulin resistance. The dissociation between hepatic lipid level and hepatic insulin resistance has been noted by others [39], and the present results add further evidence to the idea that the two processes are in fact distinct.

The mechanism behind the differential effects of sucrose and free fructose/glucose on hepatic insulin resistance cannot be determined by the present study, and the mechanisms that follow are speculative. Studies that have examined the acute effects of equal intake of sucrose or fructose/glucose solution have shown that the insulinogenic index ($\sum I/\sum G$, 0–180 min) is higher for sucrose than for fructose/glucose [40, 41]. Others have reported that acute ingestion of sucrose more effectively elevates hepatic enzymes involved in de novo lipogenesis than free fructose/glucose, including glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, acetyl CoA carboxylase and malic enzyme [42, 43]. It follows that ingestion of free fructose/glucose may lead to a greater accumulation or prolonged exposure of hepatocytes to metabolic intermediates of fructose metabolism, which promote inflammation or oxidative stress. For example, the metabolite fructose-1-P is known to activate mitogen-activated protein kinase kinase 7, which activates mitogen-activated protein kinase 8, which is thought to (indirectly) promote inflammation and induce insulin resistance [44, 45]. In this context, free fructose/glucose consumption can lead to more severe hepatic manifestations than sucrose.

The two sugar-sweetened beverage groups, on the other hand, did not differ in terms of any of the other health-related measures investigated. Notably, contrary to the results of Bocarsly et al. [20], we failed to observe a differential effect of the two sugars on body weight gain (see Table 1). As mentioned above, this was possibly a result of differences in protocols; for example, while Bocarsly used

Albino Sprague–Dawley rats, giving animals only 12-h/day access to drinks, we used Hooded Wistar rats, a pigmented rat and 24-h/day access to beverages.

As noted in the 'Introduction', a recent comparison between sucrose and fructose/glucose in chow found that the latter produced higher levels of liver triglycerides [20]. However, one major limitation of the present study, which was not found in the Sanchez et al. study, is that animals were not pair-fed. This resulted in a non-significant tendency for Group Suc to consume more total calories than Group FructSuc (see Fig. 2), and this may counteract any potentially greater effect of the fructose/glucose drink.

Behavioural findings

The current study appears to be the first to find different effects of the two sugars on locomotor activity in rats (see Table 3A). The notion that sugar can induce hyperactivity in humans has been heavily criticized [44] as only a small number of studies have reported a relationship between high sugar intake and increased activity in children [46, 47]. Although the mechanism behind the different effects of the two sugars on behaviour remains unclear, the present study suggests that type of sugar ingested needs to be considered in future investigations into a sugar's behavioural impact. Given that rats in the present study were only tested once in each environment, it would be interesting to test whether sucrose produces sustained hyperlocomotion, increased reactivity to a novel environment, or possibly both.

Conclusion

The present study supports previous findings in demonstrating excessive sweetened-drink consumption promotes the metabolic syndrome, which augments risk of diabetes mellitus, NAFLD and cardiovascular disease. These metabolic abnormalities can occur despite a rat's ability to maintain a normal body weight in the face of caloric overconsumption. Further, our results indicate 'free' fructose is more detrimental to glucose homeostasis 'bound' fructose and consequently poses a greater risk of progression to type 2 diabetes. These findings provide important evidence in support of the hypothesis put forward by Bray et al. [25], which proposes 'free' fructose, for example in HFCS, is significantly more harmful to human health than 'bound' fructose in the form of sucrose. Nevertheless, it should be noted that excessive consumption of *both* sugars promoted severe and harmful metabolic manifestations.

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References

1. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH et al (2007) Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr 86(4):899–906
2. Malik VS, Sculze MB, Hu FB (2006) Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr 84(2):274–288
3. Malik VS, Willett WC, Hu FB (2009) Sugar-sweetened beverages and BMI in children and adolescents: reanalyses of a meta-analysis. Am J Clin Nutr 89(1):438–439
4. Vartanian LR, Schwartz MB, Brownell KD (2007) Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. Am J Public Health 97(4):667–675
5. Dhingra R (2007) Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation 116(5): 480–488
6. Yoo S, Nicklas T, Baranowski T, Zakeri IF, Yang SJ, Srinivasan SR, Berenson GS et al (2004) Comparison of dietary intakes associated with metabolic syndrome risk factors in young adults: the Bogalusa heart study. Am J Clin Nutr 80(4):841–848
7. Montonen J, Jarvinen R, Knekt P, Heliovaara M, Reunanen A (2007) Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. J Nutr 137(6):1447–1454
8. Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC et al (2004) Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. JAMA 292:927–934
9. Assy N, Nasser G, Kamayse I, Nseir W, Nebiashvili Z, Djibre A et al (2008) Soft drink consumption linked with fatty liver in the absence of traditional risk factors. Can J Gastroenterol 22(10): 811–816
10. Ouyang X, Cirillo P, Sautin Y, McCall S, Brushette JL, Diehl AM et al (2008) Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol 48(6):993–999
11. Fung TT, Malik V, Rexrode KM, Manson JE, Willett WC, Hu FB (2009) Sweetened beverage consumption and risk of coronary heart disease in women. Am J Clin Nutr 89(4):1037–1042
12. Foreshee RA, Storey ML, Allison DB, Glinsmann WH, Hein GL, Lineback DR et al (2007) A critical examination of the evidence relating high fructose corn syrup and weight gain. Crit Rev Food Sci Nutr 47(6):561–582
13. Havel PJ (2005) Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev 63(5):133–157
14. Tappy L, Le KA (2010) Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 90(1):23–46
15. Almiron-Roig E, Drewnowski A (2003) Hunger, thirst, and energy intakes following consumption of caloric beverages. Physiol Behav 79(4–5):767–773
16. DellaValle DM, Roe LS, Rolls BJ (2005) Does the consumption of caloric and non-caloric beverages with a meal affect energy intake? Appetite 44(2):187–193
17. DiMeglio DP, Mattes RD (2000) Liquid versus solid carbohydrate: effects on food intake and body weight. Int J Obes 24(6):794–800

18. Bray GA (2004) The epidemic of obesity and changes in food intake: the fluoride hypothesis. Phys Behav 82(1):115–121
19. Bocarsly ME, Powelle ES, Avena NM, Hoebel BG (2010) High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. Pharmacol Biochem Behav 97(1):101–106
20. Sanchez-Lozada LG, Mu W, Roncal C, Sautin YY, Abdelmalek M, Reungjui S et al (2010) Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver. Eur J Nutr 49(1):1–9
21. Cacho J, Sevillano J, Casto De, Herrera E, Ramos MP (2008) Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Spraque-Dawley rats. Am J Physiol-Endoc M 295(5):E1269–E1276
22. Van Nieuwenhuijzen PS, Li KM, Hunt GE, McGregor IS (2009) Weekly gamma-hydroxybutyrate exposure sensitizes locomotor hyperactivity to low-dose 3, 4-methylenedioxymethamphetamine in rats. Neuropsychobiology 60(3–4):195–203
23. Moretti M, de Souza AG, de Andrade VM, Romao PRT, Gavioli EC (2010) Emotional behavior in middle-ages rats: implications for geriatric psychopathologies. Physiol Behav 102(1):115–120
24. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and it's complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539–553
25. Sclafani A (1987) Carbohydrate taste, appetite, and obesity—an overview. Neurosci Biobehav Rev 11:131–153
26. Kumari D, Nair N, Ranveer SB (2011) Effect of dietary zinc deficiency on testes of Wistar rats: morphometric and cell quantification studies. J Trace Elem Med Bio 25(1):47–53
27. Esteban-Pretel G, Marin MP, Cabezuelo F, Moreno V, Renau-Piqueras J, Timodena J et al (2010) Vitamin A deficiency increases protein catabolism and induces urea cycle enzymes in rats. J Nutr 140(4):792–798
28. Landenburg L (2006) Feast or famine: the sympathetic nervous system response to nutrient intake. Cell Mol Neurobiol 26:497–508
29. Boakes RA, Boot B, Clarke JV, Carver A (2000) Comparing albino and hooded Wistar rats of both sexes on a range of behavioral and learning tasks. Psychobiology 28(3):339–359
30. Creel D (1980) Inappropriate use of albino animals as models in research. Pharmacol Biochem Behav 12(6):969–977
31. Nakagawa T, Hu HB, Zharikov S, Tuttle KR, Short RA, Glushkova O et al (2006) A causal role for uric acid in fructose-induced metabolic syndrome. Am J Physiol-Renal 290(3):F625–F631
32. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL et al (2009) Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 119(5):1322–1334
33. Swarbrick MM, Stanhope KL, Elliott SS, Graham JL, Krauss RM, Christiansen MP et al (2008) Consumption of fructose-sweetened beverages for 10 weeks increases postprandial triacylglycerol and apolipoprotein-B concentrations in overweight and obese women. BJN 100(5):947–952
34. Teff KL, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH et al (2009) Endocrine and metabolic effects of consuming fructose and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. J Clin Endocr Metab 94:1562–1569
35. Despres JP, Lemieux I (2006) Abdominal obesity and metabolic syndrome. Nature 444(7121):881–887
36. Abdul-Ghani MA, Tripathy D, DeFronzo RA (2006) Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care 29(5):1130–1139
37. Unwin N, Shaw J, Zimmet P, Alberti KGMM (2002) Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. Diabet Med 19:708–723
38. Faeh D, Minehira K, Schwarz JM, Periasami R, Seongsu P, Tappy L (2005) Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes 54(7):1907–1913
39. Le KA, Ith M, Kreis R, Faeh D, Bortolotti M, Tan C et al (2009) Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr 89(6):1760–1765
40. Ellwood KC, Michaelis OE, Hallfrisch JG, Odorisio TM, Cataland S (1983) Blood insulin, glucose, fructose and gastric-inhibitory polypeptide levels in carbohydrate-sensitive and normal men given a sucrose or invert sugar tolerance-test. J Nutr 113(9):1732–1736
41. Shreeve WW, Hoshi M, Kikkawa R (1971) Insulin responses to ingested sucrose vs fructose in obese patients. Diabetes 20(suppl):377
42. Lee VM, Szepesi B, Hansen RJ (1986) Absence of a generalized disaccharide effect in adult female rats. J Nutr 116(8):1555–1560
43. Michaelis OE, Nace CS, Szepesi B (1975) Demonstration of a specific metabolic effect of dietary disaccharides in rat. J Nutr 105(9):1186–1191
44. Lim SJ, Mietus-Snyder M, Valente A, Schwarz J, Lustig RH (2010) The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol 7(5):251–264
45. Ruxton CHS, Gardner EJ, McNulty HM (2010) Is sugar consumption detrimental to health? A review of the evidence 1995–2006. Crit Rev Food Sci 50(1):1–19
46. Prinz RJ, Riddle DB (1986) Associations between nutrition and behavior in 5-year-old children. Nurt rev 44 (S3):151–158
47. Prinz RJ, Roberts WA, Hantman E (1980) Dietary correlates of hyperactive behavior in children. J Consult Clin Psychol 48(6):760–769