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Impact of dietary sucrose on adiposity and glucose homeostasis in C57BL/6J mice depends on mode of ingestion: liquid or solid.

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ABSTRACT

Objective: Although it is widely accepted that obesity results from an imbalance of energy intake and expenditure, the mechanisms underlying this process and effective strategies for prevention and treatment are unclear. Growing evidence suggests excess consumption of sugar may play an important role, yet we showed previously in mice that consuming up to 30% of calories as sucrose in the diet had no impact on weight regulation. Since in humans consumption of sugar-sweetened beverages has been widely implicated, we investigated whether the mode of ingestion (solid or liquid) had different impacts on body weight regulation and glucose homeostasis.

Methods: Dietary sucrose was delivered in solid (as part of a standard pelleted rodent chow) and liquid (in drinking water) to C57BL/6 mice for 8 weeks. Body weight, body composition, energy intake, and expenditure were monitored, and glucose and insulin tolerance tests were given. Expression of sweet taste receptors on the tongue and glycogen and fat contents of the liver were also measured.

Results and conclusions: Consumption of sucrose-sweetened water, but not equivalent levels of solid sucrose, led to body fat gain in C57BL/6 mice. Glucose intolerance was positively correlated to body fatness rather than sucrose intake. Our data support the suggestion that consumption of liquid sucrose may be an important contributor to dysregulation of body weight and related metabolic syndromes.

Key words: Dietary sucrose; sweet taste receptors; Glucose tolerance; Insulin sensitivity; Obesity.

1. INTRODUCTION

Obesity, diabetes, and other metabolic related disorders remain on the rise globally [1-3]. It is widely agreed that the main cause of obesity is an imbalance between energy intake and energy expenditure [4-6]. It is widely disagreed, however, which of these is the most important and the details of why intake may have increased or expenditure declined. Although early work implicated reduced expenditure as the key driver [7] more recent direct measurements of expenditure suggest no decline in energy demands over the time course of the obesity epidemic [8]. In contrast, the expanding food supply can more than account for the increased obesity levels [9]. However, while elevated food supply is likely the most significant key driver of the epidemic, the components of the diet that cause elevated intake are disputed, with different researchers favoring elevated fat consumption [10, 11], elevated refined carbohydrates [12], or reduced protein intake [13, 14].

One particular focus of attention has been the consumption of sucrose [15, 16], notably in the form of sugar-sweetened beverages [17]. A major problem with these epidemiological studies, however, is that they rely on correlation to imply causative effects. However, the negative impacts of diet on health mean that it is ethically challenging to perform randomized controlled trials in humans to establish what the macronutrient drivers of excess body adiposity actually are. To this end, rodent models may provide useful translational insights into dietary impacts on weight regulation and metabolic homeostasis. We recently performed such a study using 5 different mouse strains exposed to 29 different diets including more than 1000 individual mice and over 100,000 measurements of body weight [11]. This work indicated that the only factor driving excess calorie consumption and adiposity was elevated fat in the diet. Surprisingly, we found that changing the sucrose content between 5 and 30% did not affect weight gain when fat and protein levels were kept constant. This result contrasts with earlier work in rodents [17] in which sucrose was provided in the drinking water, and this did cause an increase in adiposity. The reasons for the differences in the outcomes of these experiments are unclear. On one hand, the mode of delivery of the sucrose may be a factor. On the other hand, when

sucrose was provided in the water, the total intake of sucrose as a % of the total calories (c 70%) was much higher than the maximum 30% that was used by Hu et al. [11]. Thus, it might be that if Hu et al had used a diet with 70% sucrose in the pelleted diet they would have found a similar effect. Which of these explanations is correct is important, because if the mode of delivery of the sugar, rather than the amount, is the main factor, this would support the suggestion that sugar sweetened beverages are a potential driver of the obesity epidemic [18-21]. In the current paper, we aimed to resolve whether mode of sucrose delivery is a factor affecting the adiposity response of C57BL/6J mice.

We found that liquid sucrose exposure contributed to higher energy consumption leading to greater body weight and body fat. Mice exposed to equivalent levels of sucrose in the solid diet were leaner and metabolically healthier than their counterparts exposed to liquid sucrose. Animals accessing liquid sucrose displayed blunted insulin sensitivity and higher expression of hepatic *IL-6*. Sensitivity to IP glucose and insulin was negatively affected by body fatness. Increased liver size in mice drinking sucrose water was associated with more fat storage rather than elevated glycogen as determined by direct quantification and expression of glycogen and fat storage related genes. Together these studies suggest an important impact of mode of sucrose delivery, and new details of mechanisms underlying sugar-sweetened beverage consumption relevant to the current obesity epidemic.

2. MATERIALS AND METHODS

2.1 Diets

In a pilot study that lasted for 8 weeks, 2 groups of mice were fed one of the following diets. The first group was exposed to a control diet consisting of 20%kcal from fat, 25%kcal from protein and 30%kcal from sucrose (in 55% total carbohydrates). A second group of mice was fed the control diet and also given free access to sucrose-sweetened water (50% by weight) without access to other drinking water. We also investigated food preference of these animals for the solid sucrose diets. Diets F30 and F73 (see below) were simultaneously available on each side of the animals feeding cage, and intake of each diet was measured daily for 6 days.

In the main study (based on the results of the pilot study), mice were assigned into one of the four dietary treatments. Details of the experimental diets are displayed in Supplementary Table 1. Briefly, 10 mice were exposed to low fat diet with free access to water that contained no sucrose with 25%kcal from protein, 20%kcal from fat, and 55%kcal from carbohydrate (30%kcal from sucrose) (referred to as diet F30/W0: the F number refers to the % sucrose in the food and the W refers to the sucrose % in the water). A second group (n = 10) was exposed to the same diet but the water bottle was replaced by sucrose solution (50% by weight) and will be referred as diet F30/W50. A third group (n = 10) was given the same treatment as the second, but this group was also given free access to both water and the sucrose solution in two separate bottles and will be referred as diet F30/W50/W0. The fourth group (n = 10) was given access to a diet that was formulated to mimic the macronutrient intake of the second group based on the pilot study. This diet was composed of 10% energy from protein, 8% energy from fat and 82% energy from carbohydrate (of which 88.6% of the carbohydrate energy was from sucrose = 73% of total energy) and will be referred as diet F73/W0 (Supplementary Table 1). The dietary treatment was continued for a period of 8 weeks, following a 2-week baseline period.

2.2 Animals

Animal experiments were approved by the animal ethical committee of the Institute of

Genetics and Developmental Biology, Chinese Academy of Sciences (Beijing, China) approval number AP2016039.

Male C57BL/6J mice (8 weeks of age) were purchased from Charles River Laboratories and individually housed in pathogen free conditions at room temperature (23 ^{o}C) with 12 h light/dark cycle. All mice were fed a standard diet with 10% fat and 20% protein, 35% sucrose (D12450B, Research Diets Ltd) for 2 weeks as the baseline period prior to the dietary treatment. Body weight, food, and liquid sucrose intake were measured daily. Food intake was obtained by subtracting remaining food in the hopper, including any spilled food in cages, from the previous days weighed aliquot. Energy intake was calculated based on caloric values obtained from Research Diets. An EchoMRI Body Composition Analyzer was used to measure body composition including fat mass and lean mass [22] once a week over the 8 week period following 2 weeks of baseline measurement. Canola oil was used as the standard for the measurements. At the end of the study, all mice were sacrificed, and fresh tissues were immediately frozen for analysis. Soxhlet (XMTD-7000, Changhai) was used to extract lipid of dry liver tissue to provide a quantitative measure of hepatic fat content. Hepatic glycogen content was determined using a commercially available kit (Cat # E2GN-100, EnzyChrom, BioAssay Systems, U.S.A).

2.3 Energy Expenditure and Physical Activity measurement

After 6 weeks of dietary exposure, mice were put into a TSE PhenoMaster/LabMaster system for 3 consecutive days, sufficient to obtain an accurate measure of energy metabolism [23]. Using this system, we recorded different parameters such as the oxygen (O_2) consumption (mL/min), carbon dioxide (CO_2) production (mL/min), respiratory exchange ratio (RER = VCO_2/VO_2), locomotor activity (Counts/s), food intake (g) as well as water and sucrose intake. Measurements were taken at 6-min intervals for the whole period. Daily Energy expenditure (DEE) was calculated from O_2 consumption and CO_2 production according to the Weir Equation: EE (kJ/day) = ((3.9 x VO_2 (mL/min) + 1.1 x VCO_2 (mL/min)) x 1440 (min)/1000 x 4.184 [24].

To determine energy assimilation efficiency, food intake and feces production were

daily monitored in mice singly housed for 3 days on the week 8 of the dietary exposure. Bomb calorimetry (Parr 1281 bomb calorimeter) was used to analyze feces samples for their energy content.

2.4 Blood parameters

A glucose tolerance test was performed on the 6th week of diet exposure by intraperitoneal (I.P.) injection of glucose at 2 g/kg of body weight following a 14 h fast, and circulating glucose levels were measured *in vivo* [25]. Blood samples were taken from the tail vein at 0, 15, 30, 60, and 120 min after injection and blood glucose was determined with an OneTouch ultravue^{TM} glucometer (Changsheng, China). For the insulin sensitivity test, animals were intraperitoneally injected with Humulin R insulin (Novolin R, Novo Nordisk) at 0.75 U/kg of body weight following a 4 h fast. Blood samples were taken from the tail vein at 0, 15, 30, 60, and 120 min after injection. Blood glucose levels were plotted against time, and the area under the curve was calculated. Fasting serum insulin levels were quantified using the Ultra Sensitive Mouse Insulin ELISA (Crystal Chem, Cat # 90080, Elk Grove Village, IL, U.S.A.). The homeostatic model assessment (HOMA-IR), most commonly used to assess the degree of insulin resistance and glucose intolerance was determined using a modified equation described by Vasques and colleagues [26]. Quantitative colorimetry (EnzyChrom^{TM} EFRU-100, BioAssay Systems) was used to determine fructose level in serum samples.

2.5 Total RNA extraction, cDNA synthesis and real-time RT-PCR

Mouse liver and tongue tissues were immediately frozen in liquid nitrogen upon sacrifice. Homogenization was performed in a 2 ml PCR-PT microtube (SARSTEDT AG and Co.KG, Numbrecht, Germany) using Omni bead ruptor 24 homogenizer (Kennesaw GA, 30144 United States) with stainless steel beads. Extraction of RNA, cDNA synthesis and transcript analysis have been previously described in detail [27]. Briefly, total RNA was extracted from frozen tissues using Tri-Reagent (Tri-Reagent, Mei Biotechnology, Co. Ltd, China). First strand cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen) and random hexamers. Quantitative

polymerase chain reaction (qPCR) was performed using the 2x realtime PCR mix (SYBRgreen). PCR primers are listed in supplementary table 3. Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) was used for normalization. Relative quantitation of transcript levels was analyzed based on the comparative cycle threshold method $2^{-\Delta Ct}$ with Ct values obtained from PCR kinetics measured by the Roche LightCycler® 480 Real-Time PCR.

2.6 Statistical analysis

Data were analyzed using GraphPad Prism 6.0 software (La Jolla, CA, USA). Tissues were weighed to the nearest 0.01g. Expression of mRNA, tissue mass data, and metabolic parameters (area under the curve, insulin and glycogen) were analyzed by One-way ANOVA with posthoc Tukey's test. Expression data were log-transformed before analyzing to approximate a normal distribution. Body mass data, ITT, and GTT were analyzed using 2-way ANOVA with repeated measures (RM) followed by posthoc Sidak test. Correlations were determined using Pearson's correlation coefficient. Analysis of covariance (ANCOVA) was performed for oxygen consumption and energy expenditure data [28, 29]. To fit regression models to the individual data, we first used a linear model and then explored the distribution of the residuals in relation to the predictor variables. If these were clearly structured and not random, we fitted non-linear models until the residual distribution was random. All statistical tests were applied as indicated and $p < 0.05$ was considered significant. Data are plotted as mean ± S.E.M

3. RESULTS

3.1 Pilot study

Mice with access to liquid sucrose (F30/W50) had significantly higher body weight compared to the control group (F30/W0) (paired *t*-test, $p < 0.001$, Supplementary Figure 1A). In addition, access to liquid sucrose led to a significant reduction in the solid food intake compared to the water group (paired *t*-test, $p < 0.001$,

Supplementary Figure 1B). We compared the caloric intake from food alone for the F30/W0 group and from the food plus liquid sucrose in the (F30/W50) group. Access to liquid sucrose led to greater overall caloric intake compared to the group with access only to solid food (paired *t*-test, $t = 19.57$, $p < 0.001$, Supplementary Figure C). We calculated the intakes of carbohydrate (sucrose), protein and fat in the F30/W50 group (by energy), and this indicated they were consuming 10% protein, 8% fat and 82% carbohydrate (of which 73.1% of the total intake was sucrose) (Supplementary Table 2). We then used this formulation to design a new solid diet that mimicked the combination of liquid and solid intake in the F30/W50 group. This diet called F73/W0. In the food preference tests comparing the F30/W0 and F73/W0 diets (supplementary Figure 2) when given a choice the mice preferred to consume more of the F30 than the F73 solid diet (paired t-test, $t = 3.586$, $p = 0.015$)

3.2 Liquid sucrose contributes to body weight gain

Body weight and body fat were significantly greater in both groups of mice that had access to liquid sucrose (ANOVA, $F_{3, 2268} = 552.2$, $F_{3, 324} = 74.16$, $p < 0.0001$, Figure 1A and B, respectively). Mice fed F30/W50 and F30/W50/W0 diets had significantly lower solid food intake throughout the treatment (2way ANOVA, $F_{3, 2232} = 144$, $p < 0.0001$, Figure 1C). Comparing the two groups with access to liquid sucrose, mice fed the F30/W50 diet had significantly higher liquid sucrose intake compared to the F30/W50/W0 group, which had a choice between water and liquid sucrose ($p < 0.0001$, Figure 1D). The liquid sucrose fed mice therefore had significantly lower energy intake from solid diet compare to the control group and the F73/W0 fed mice (2way ANOVA, $F_{3, 1836} = 1456$, $p < 0.0001$, Figure 1E). However, their energy intake from liquid sucrose was higher than that from their solid food. Moreover, treatment F30/W50 had significantly higher liquid sucrose energy input compared to F30/W50/W0 (paired *t*-test, $t = 10.54$, $p < 0.0001$, Figure 1F). Nevertheless, both of these groups of mice had significantly higher absolute sucrose intake compared to the mice fed only solid food F30/W0 and F73/W0 (2way ANOVA, $F_{3, 2196} = 1066$, $p < 0.0001$, Figure 1G). It is noteworthy that the group treated with F73/W0 was the

leanest, but had significantly higher absolute sucrose intake compared to the F30/W0 fed group (paired *t*-test, t = 16.29, p < 0.0001). The overall energy intake was significantly higher in liquid sucrose fed groups compared to solid diet fed F30/W0 and F73/W0 (2way ANOVA, $F_{3, 2196}$ = 229.9, p < 0.0001, Figure 1H)**.**

The reduced solid intake when drinking sucrose suggested the mice were attempting to regulate their total caloric intake in response to their liquid sucrose intake, but failing to do so. To understand the increased fat mass in both liquid sucrose groups, we assessed energy balance in all animals by indirect calorimetry. Oxygen consumption and daily energy expenditure (ANOVA, $F_{3, 36}$ = 10.98, p < 0.0001) were significantly different among treatments. In particular these were significantly higher in F30/W50 fed mice compared to the F73/W0 group (paired *t*-test, p < 0.05). However, when ANCOVA was used to adjust for body weight effect, DEE was not significantly different among groups (p = 0.214, Figure 2A and B). Food intake displayed a normal nocturnal pattern in all groups with the respiratory exchange ratio higher during night time in all groups compared to day time (paired *t*-test, t = 19.43, p < 0.0001). Furthermore, this ratio was significantly higher in the F30/W50 fed mice in day time compared to F30/W0 and F73/W0 groups for the same period (ANOVA, $F_{3, 36}$ = 4.24, p = 0.011). However, during night time, F73/W0 had the highest RER compared to the other groups (ANOVA, $F_{3, 36}$ = 4.66, p = 0.0075, Supplementary Figure 3). When ambulatory activity was assessed, we found that mice were more active during the dark period (paired *t*-test, t = 15.31, p < 0.0001). In particular the F30/W0 group was marginally more active compared to the F30/W50 group (*t*-test, p = 0.054,) during night time. However, overall activity was not different when compared across all groups (2way ANOVA, $F_{3, 36}$ = 0.29, p = 0.82 and $F_{3, 36}$ = 0.71, p = 0.55 for day and night activity respectively, Figure 2C), which suggested that the increased body fat in liquid sucrose groups was not a result of lowered physical activity. F73/W0 mice produced the least feces and had the highest assimilation efficiency compared to the F30/W0 mice and those with access to liquid sucrose (ANOVA, $F_{3, 24}$ = 26.54, p < 0.0001, Figure 2D). Assimilation efficiency was lowest in the two groups with access to liquid sucrose, with no significant difference between

these two groups.

3.3 Liquid sucrose contributed to hepatic fat accumulation

Liver wet weight was significantly greater in both groups presented with sucrose in the drinking water compared to those fed solid foods (ANOVA, $F_{3, 34} = 10.11$, $p < 0.0001$, Figure 3A). However, no difference was noted between F30/W50 and F30/W50/W0 fed groups (*t*-test, $p = 0.29$). The liver weight to body weight ratio was also significantly higher in both liquid sucrose groups and was positively correlated with body weight (ANOVA, $F_{3, 34} = 5.86$, $p = 0.0024$, Figure 3B). We evaluated whether this difference in liver weight was due to either glycogen or fat. The liver glycogen level was not significantly different among all 4 groups ($F_{3, 34} = 0.397$, $p = 0.75$, Figure 3C). To confirm this result, we quantified mRNA expression of *G6pase*, a gene that is primarily involved in glycogen metabolism. No significant difference was noted in *G6pase* expression among all groups (ANOVA, $F_{3, 33} = 0.13$, $p = 0.93$, Figure 3D). Concerning lipid metabolism, no significant difference in FAS expression was observed among groups (ANOVA, $F_{3, 33} = 0.70$, $p = 0.55$, Figure 3E). However, *PPARγ* expression was significantly upregulated in both liquid sucrose fed groups compared to mice exposed to the F30/W0 and F73/W0 diets (ANOVA, $F_{3, 33} = 7.708$, $p < 0.001$, Figure 3F). When total lipid was determined, we found that mice presented sucrose in the drinking water had significantly higher hepatic lipid content compared to the ones fed solid sucrose diets (ANOVA, $F_{3, 33} = 13.47$, $p < 0.0001$, Figure 3G). However, there was no significant difference between the F30/W50 and F30/W50/W0 diets. Together, these results suggest that liquid sucrose intake drove elevated calorie intake leading to increased liver fat storage, but exposure to the same percentage of sucrose via a solid diet did not. We measured a marker of inflammation in liver to further elucidate the deleterious effect on liquid sucrose. Expression of *IL-6* mRNA was found higher in particularly the mice fed F30/W50 compared to all 3 groups. Furthermore, this difference was statistically significant compared to the control F30/W0 group (*t*-test, $p = 0.017$).

3.4 Liquid sucrose altered glucose homeostasis

To explore the relationship between body fat, diet, and glucose homeostasis, we performed *in vivo* glucose tolerance tests. We found there was a significant effect of diet on the glucose homeostasis (2-way ANOVA, $F_{3, 180} = 10.59$, $p < 0.0001$, Figure 4A). Mice fed F73/W0 had significantly better glucose tolerance compared to all of the 3 other treatments. This was also supported by the AUC analysis (ANOVA, $F_{3, 36} = 3.705$, $p = 0.02$, Figure 4B). However, no significant difference was noted between both liquid sucrose F30/W50 and F30/W50/W0 (*t*-test, $p = 0.18$) along with the F30/W0 fed groups (ANOVA, $F_{2, 27} = 0.81$, $p = 0.45$, Figure 4B).

Furthermore, liquid sucrose led to a significantly lower response to insulin when compared to the solid sucrose fed groups F30/W0 and F73/W0 (2-way ANOVA, $F_{3, 138} = 26.11$, $p < 0.0001$, Figure 4 C). This was also confirmed when AUC analysis was performed (ANOVA, $F_{3, 28} = 9.38$ $p = 0.0002$, Figure 4D). Mice fed the F73/W0 diet were particularly sensitive to insulin in comparison to the F30/W50 and F30/W50/W0 treatment groups. We also evaluated the fasting serum level of insulin at sacrifice. As expected, circulating insulin levels were significantly higher in treatments F30/W50 and F30/W50/W0 (ANOVA, $F_{3, 33} = 8.70$, $p = 0.0002$, Figure 4 E). This increase in serum insulin level indicated an impaired peripheral insulin sensitivity in both liquid sucrose fed groups. However, the F30/W50 was not significantly different to the F30/W50/W0 group (*t*-test, $p = 0.14$).

In parallel, we tested whether the sustained lower response to insulin was coupled to a decrease in hepatic insulin receptor-mediated inhibition of insulin signaling, resulting in higher blood glucose. We found that F73/W0 fed mice had significantly higher mRNA expression of *Irs2* (ANOVA, $F_{3, 33} = 3.20$, $p = 0.03$, Figure 4F). These data imply that the reduced glucose tolerance was linked to impaired insulin signaling. We performed correlation tests to determine the cause of the disturbance in glucose metabolism.

3.5. High solid sucrose intake, not liquid, induces the upregulation of lingual sweet taste receptors (*Tas1r2* and *Tas1r3*)

In view of the key potential roles of sweet taste receptors and their influence on food

intake, we measured the lingual expression of these to understand the increase in energy intake and consequent body weight gain when fed liquid sucrose.

We found that the high solid sucrose diet F73/W0 induced significant upregulation of lingual mRNA expression of the *Tas1r2* and *Tas1r3* genes (ANOVA, $F_{3, 31} = 9.49$, $p < 0.0001$ and $F_{3, 31} = 3.62$, $p = 0.02$, Figure 5A and B, respectively). In contrast, expression of these receptors was marginally reduced in treatment F30/W50 compared to control F30/W0. The upregulation of Tas1r2 and Tas1r3 in the leaner mice was accompanied by functional improvement in glucose metabolism because mice on the F73/W0 had significantly higher capacity of glucose clearance following glucose injection, higher sensitivity to insulin load and had lower plasma insulin level. These results together imply that the changes in the metabolic parameters cannot be attributed to dietary sucrose intake but rather to body weight/fatness, suggesting only an indirect link between STRs signaling and body fatness.

We found that this altered metabolic homeostasis was mostly attributable to body fatness rather than directly to energy input from sucrose. There was a positive correlation of body weight ($R^2 = 0.191$, $p = 0.004$) and body fat ($R^2 = 0.174$, $p = 0.007$) with blood glucose level (Figure 6A and B respectively). This was also strongly supported by a positive correlation between serum insulin level and body weight ($R^2 = 0.771$, $p < 0.0001$, Figure 6C). This increased serum insulin level was negatively associated with lower hepatic expression of Irs2 (Figure 6F). The impaired insulin response in liquid fed groups was also consistent with elevated plasma fasting insulin and HOMA-IR values (ANOVA, $F_{3, 34} = 6.17$, $p < 0.01$, Figure 6E). However, there was no significant association between blood glucose level and energy intake from sucrose ($R^2 = 0.02$, $p = 0.39$, Figure 6D). Together, these data imply a negative impact of sugar consumed in liquid form on glucose homeostasis and insulin.

4. DISSCUSSION

In the current study, we sought to assess the impact of the mode of sucrose delivery on energy balance, adiposity, and glucose homeostasis in mice. Recently, we demonstrated [11] that dietary fat was the main factor that causes mice to gain weight.

We showed that dietary sucrose treatment did not have any significant influence on energy intake and body weight in C57BL/6 mice, but the range of sucrose levels used was limited (5 to 30%) and it was only delivered in solid form as a component of the diet. Previous work has suggested that sucrose in the drinking water may lead to adiposity in rodents [17]. The cause of this difference is unclear. It could be because the level of ingested sucrose when delivered in water is much higher (about 73% by calories) or because there is something special about delivering the sucrose in liquid as opposed to solid form. The current results demonstrate that when exposed to liquid sucrose, mice had greater energy intake than when offered the same macronutrient composition but in solid form. Furthermore, these mice did not have significantly elevated energy expenditure in response to the increased caloric input. This led to greater adiposity and impaired blood glucose homeostasis and insulin resistance compared with the F73/W0 fed group. This protection was primarily, because mice exposed to the F73/W0 condition had much lower total energy intake. These mice also had lower total energy intake than mice on the control F30/W0 diet, and in preference tests (supplementary Figure 2) the mice preferred the F30 to the F73 diet. The reasons for this preference may be related to the other macronutrient differences between the F30 and F73 diets. Hence while the F73 diet had much more sucrose it had correspondingly less fat and protein. These other macronutrients (particularly fat) may have driven the preference.

A number of studies suggested that weight gain may occur because compensation at subsequent meals for energy consumed in the form of a liquid may be less complete than that for energy consumed in the form of a solid, most likely because of the low satiety of liquid foods [30]. For example, DiMeglio and Mattes [31] showed that consumption of 1180 kJ soda/d resulted in significantly greater weight gain than consumption of an isocaloric solid carbohydrate load. Others have reported similar findings [32-35]. Many human studies have shown a connection between consumption of sugar-sweetened beverages and total energy intake [30, 36], which suggests that when persons increase liquid carbohydrate consumption, they do not concomitantly reduce their solid food consumption [33, 37]. In the present study,

consumption of liquid sucrose concomitantly reduced solid food intake to some extent. However, this reduction was insufficient to balance the elevated calorie intake in the liquid sucrose. These data therefore support the suggested role of sugar-sweetened beverages in the development of diet-induced obesity and insulin resistance. Liquid sucrose feeding led to a significantly higher fat accumulation in the liver compared to the same level (%) of solid sucrose in the diet. However, this difference could reflect the different absolute sucrose intakes. Direct measurements of glycogen levels and expression of the glycogen metabolism marker *G6pase* in liver did not indicate an accumulation of glycogen in the liver. However, extraction of total lipid content in liver and measures of fatty acid metabolism related genes and pro-inflammatory *IL-6* mRNA did show altered hepatic fat metabolism. The presence of excessive hepatic fat levels in liquid sucrose fed groups might be causally linked to the impaired glucose homeostasis compared with F73/W0. Glucose intolerance and insulin resistance are known to be independent and additive risk factors for the development of metabolic disorders such as type 2 diabetes and cardiovascular disease [38, 39]. In conjunction with the increase in adiposity and hepatic inflammation described above, we also observed an impairment of glucose homeostasis in the groups fed liquid sucrose, relative to those exposed to the same sucrose percentage but in solid form.

Because the mice feeding in the F30/W50 and F30/W50/W0 conditions had higher absolute sucrose intake than those in the F73/W0 condition it might be argued that their poorer performance in the GTT and ITT relative to those on the F73/W0 diet was a consequence of their higher absolute sucrose intake. However, this did not appear to be the case, because AUC for both the GTT and ITT were unrelated to absolute sucrose intake, and much more closely linked to body weight and body fatness (Figure 6). An unexpected outcome from these data was the protection afforded by eating the F73/W0 diet. In fact, although the mice in the F30/W50 condition had greater body weight gain and impaired GTT and ITT compared to mice eating F73/W0, they did not differ from the mice eating the control diet F30/W0. This comparison, however, is confounded by the fact that the components of the diet are different between these groups. Hence, both F30/W50 and F73/W0 groups had both

lower fat and lower protein intakes than the F30/W0 mice. The relative protection of the F73/W0 diet may then be because of the lower levels of intake of these other macronutrients. This raises the question then why the F30/W50 mice were not similarly protected, and the answer may be that any benefits were offset by the liquid sucrose intake.

The mechanisms underlying the different responses of the mice to solid and liquid sucrose at present remain unclear. A recent paper showed that when *Drosophila* were exposed to sucrose in their drinking water, there was a strong downregulation of sweet taste receptors, and this blunted sensitivity led to overconsumption of the sucrose water [40]. Although the taste receptors are different in mice and *Drosophila*, we can reject this possible mechanism, because our measurements of sweet taste receptors *Tas1r2* and *Tas1r3* of mice exposed to sucrose water showed no change (Fig 5). However, there was significant upregulation of these receptors in mice exposed to high levels of solid sucrose (discussed further below), and this hypersensitivity might be linked to the lower consumption of this diet. In addition, it seems likely that the dynamics of sucrose digestion and the uptake of the resultant glucose and fructose molecules in the small intestine is different for the solid and liquid diets. These different dynamics of changes in post-prandial glucose and fructose levels may then exert different impacts on the hypothalamic gene expression that governs hunger and food intake: with liquid intake having a more muted effect on satiety. The mechanism underlying the altered insulin sensitivity also remains uncertain. While we measured levels of *Irs2* and showed these were reduced in the mice exposed to liquid sucrose a much more expansive treatment of this topic is required to more fully understand the mechanisms involved.

The preference for sweet taste is partially genetically determined [41]. The major allele of the single nucleotide polymorphism rs12033832 in the sweet taste receptor (*Tas1r2*) has previously been associated with lower sugar sensitivity and higher sugar intake among overweight individuals [42]. Taste in mammals provides sensory information that helps in evaluating food nutritional qualities, food selection, and

dietary intake. Therefore, they are an important component of the whole food intake regulation system. Obesity has been reported to decrease expression of *Tas1r3* and *in vitro* high levels of glucose have been shown to cause down-regulation of *Tas1r2* [43]. The lower circulating glucose at all points in time as shown by GTT and ITT in the F73/W0 group, therefore, may be a factor involved in the upregulation of the *Tas1r2* and *Tas1r3* genes in the lean mice fed the F73/W0 diet.

5. CONCLUSION

In conclusion, our study indicates that the mode of dietary sucrose delivery has a significant impact on regulation of body composition in C57BL/6J mice. Sucrose consumption in solid form, even when comprising 73% of ingested calories, did not lead to elevated food intake and did not induce elevated adiposity. Consequently, mice fed solid sucrose were leaner and metabolically healthier. In these mice, high solid sucrose intake led to an upregulation of sweet taste receptors (*Tas1r2* and *Tas1r3*). However, the same amount of sucrose given in liquid form was responsible for greater body weight gain and increased adiposity as well as an accumulation of fat in the liver. The expression of the hepatic insulin receptor substrate 2 was repressed, correlated with a higher serum insulin level. These, in turn, were related to impaired insulin action and perturbed glucose homeostasis. Sugar only had a negative impact on glucose homeostasis when it caused elevated adiposity. The present work strongly supports the suggestion that sugar-sweetened beverages may be important drivers of adiposity and thereby impaired metabolic health.

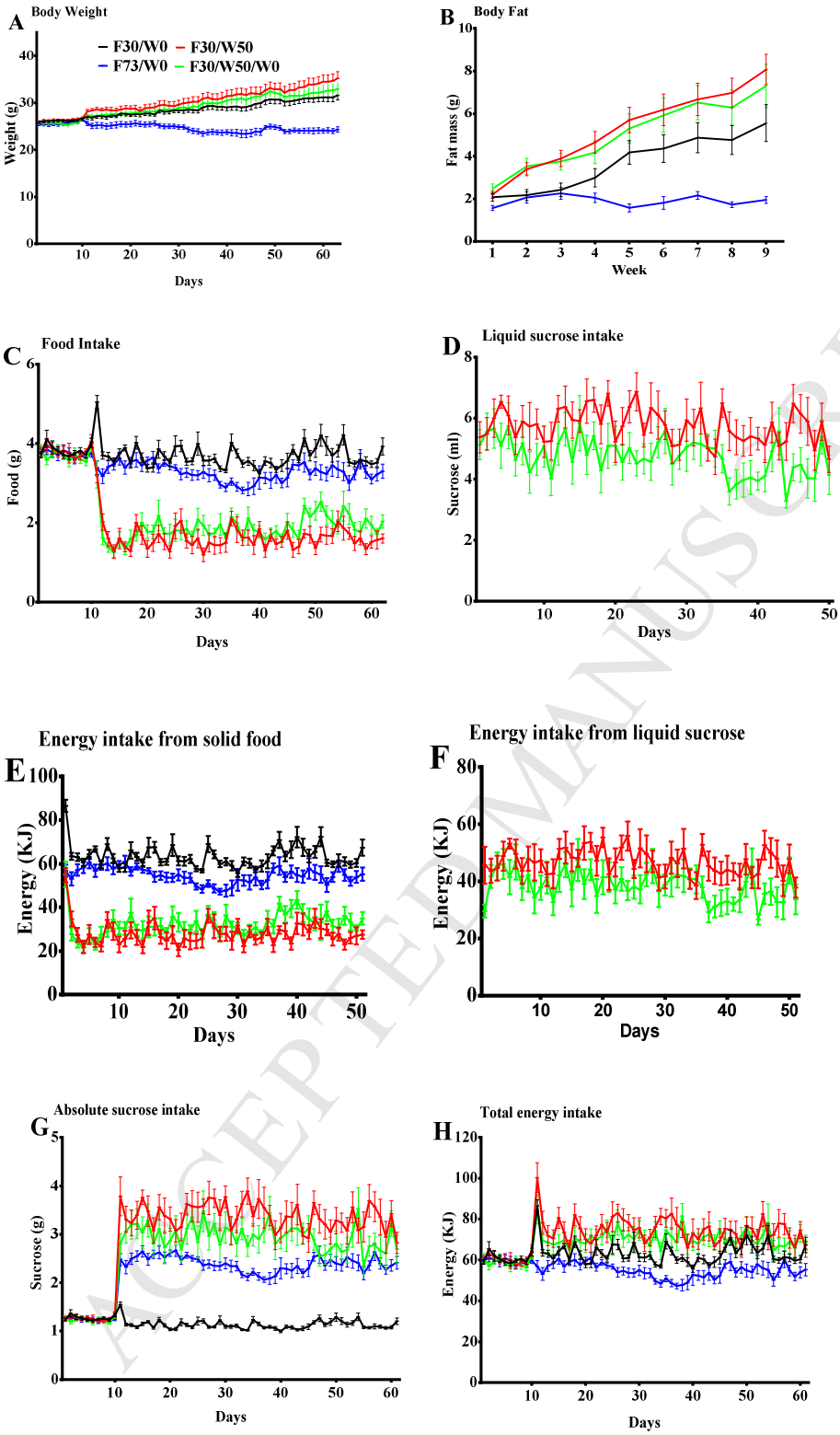

Figure 1

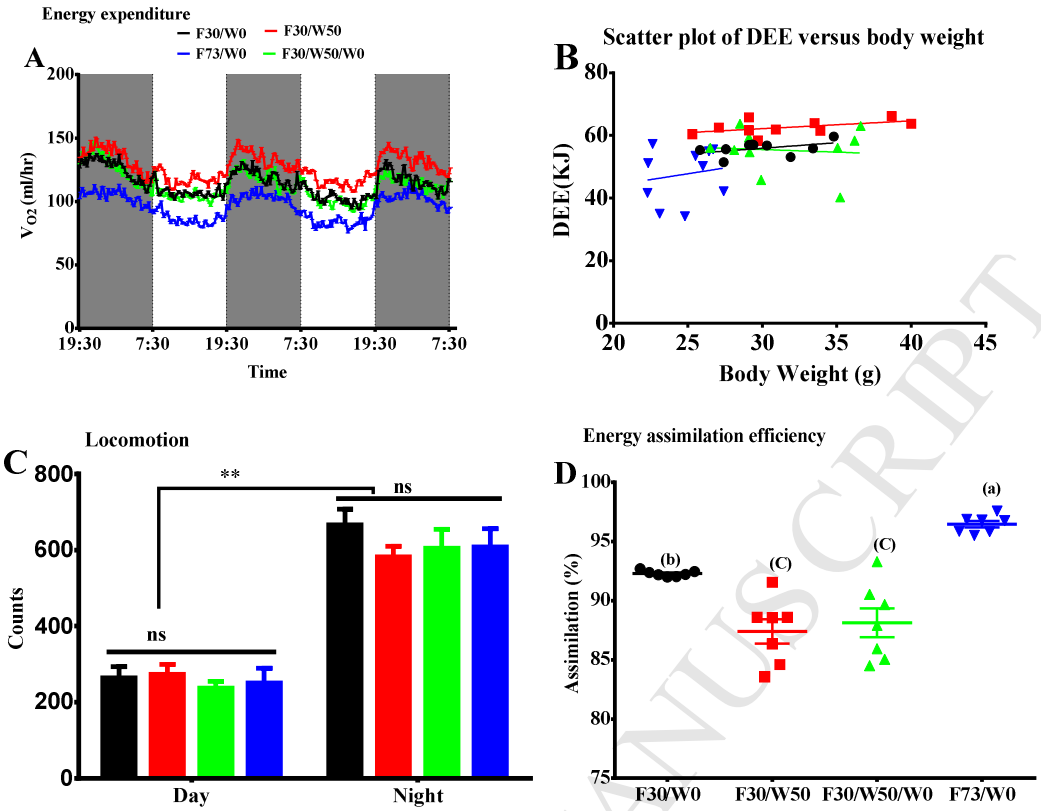

Figure 2

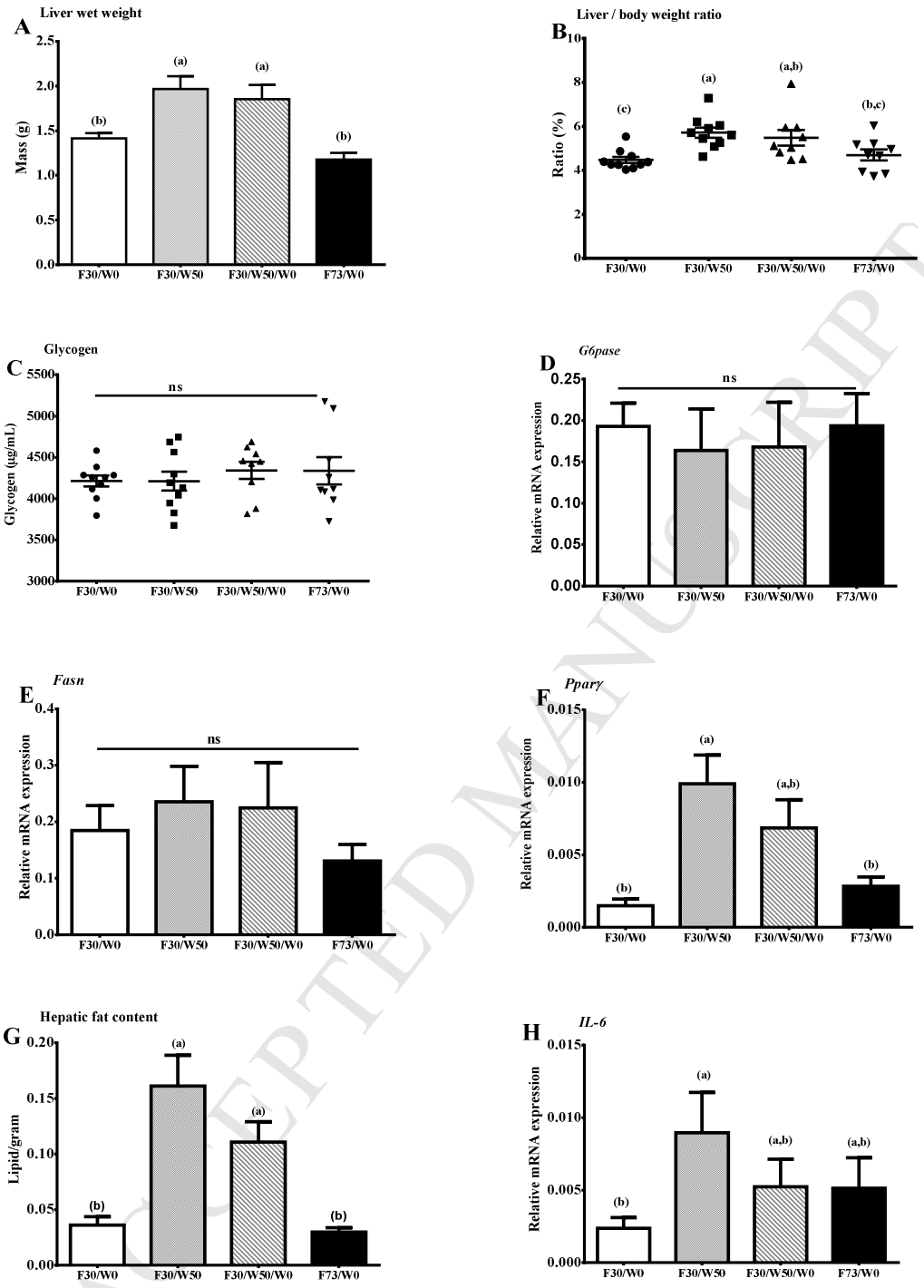
A
Liver wet weight
Mass (g)
(b)
(a)
(a)
(b)
F30/W0
F30/W50
F30/W50/W0
F73/W0
B
Liver / body weight ratio
Ratio (%)
(c)
(a)
(a,b)
(b,c)
C
Glycogen
Glycogen (µg/mL)
ns
D
G6pase
Relative mRNA expression
ns
E
Fasn
Relative mRNA expression
ns
F
Pparγ
Relative mRNA expression
(b)
(a)
(a,b)
(b)
G
Hepatic fat content
Lipid/gram
(b)
(a)
(a)
(b)
H
IL-6
Relative mRNA expression
(b)
(a)
(a,b)
(a,b)

Figure 3

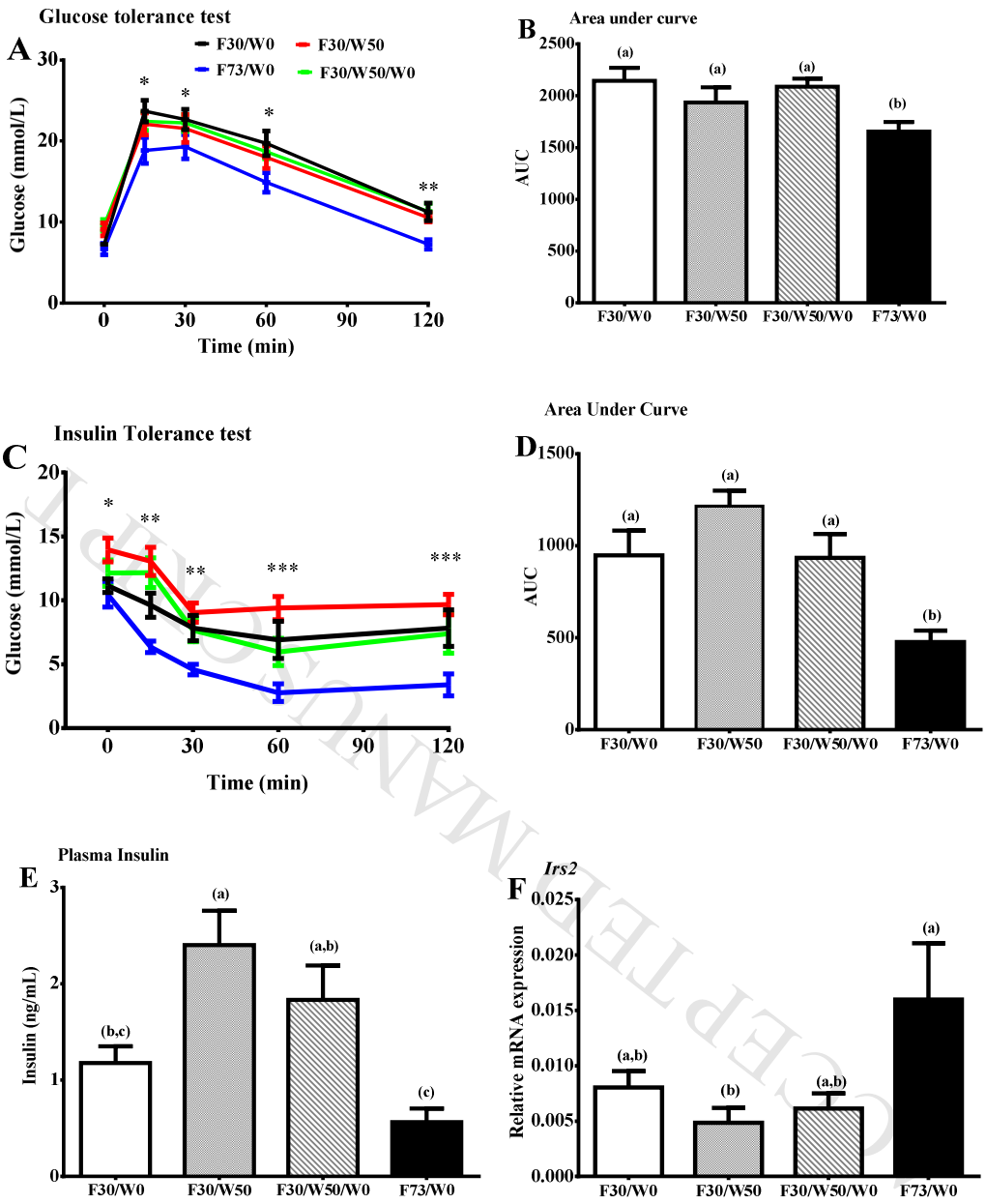

Figure 4

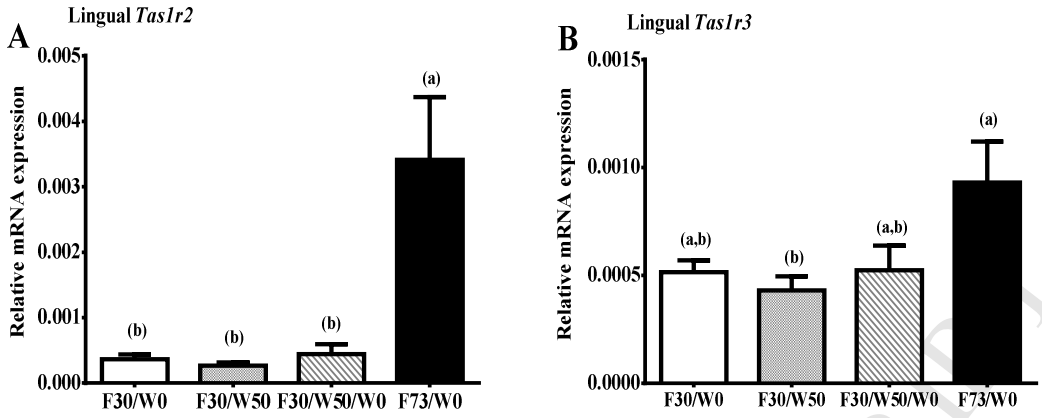

Figure 5

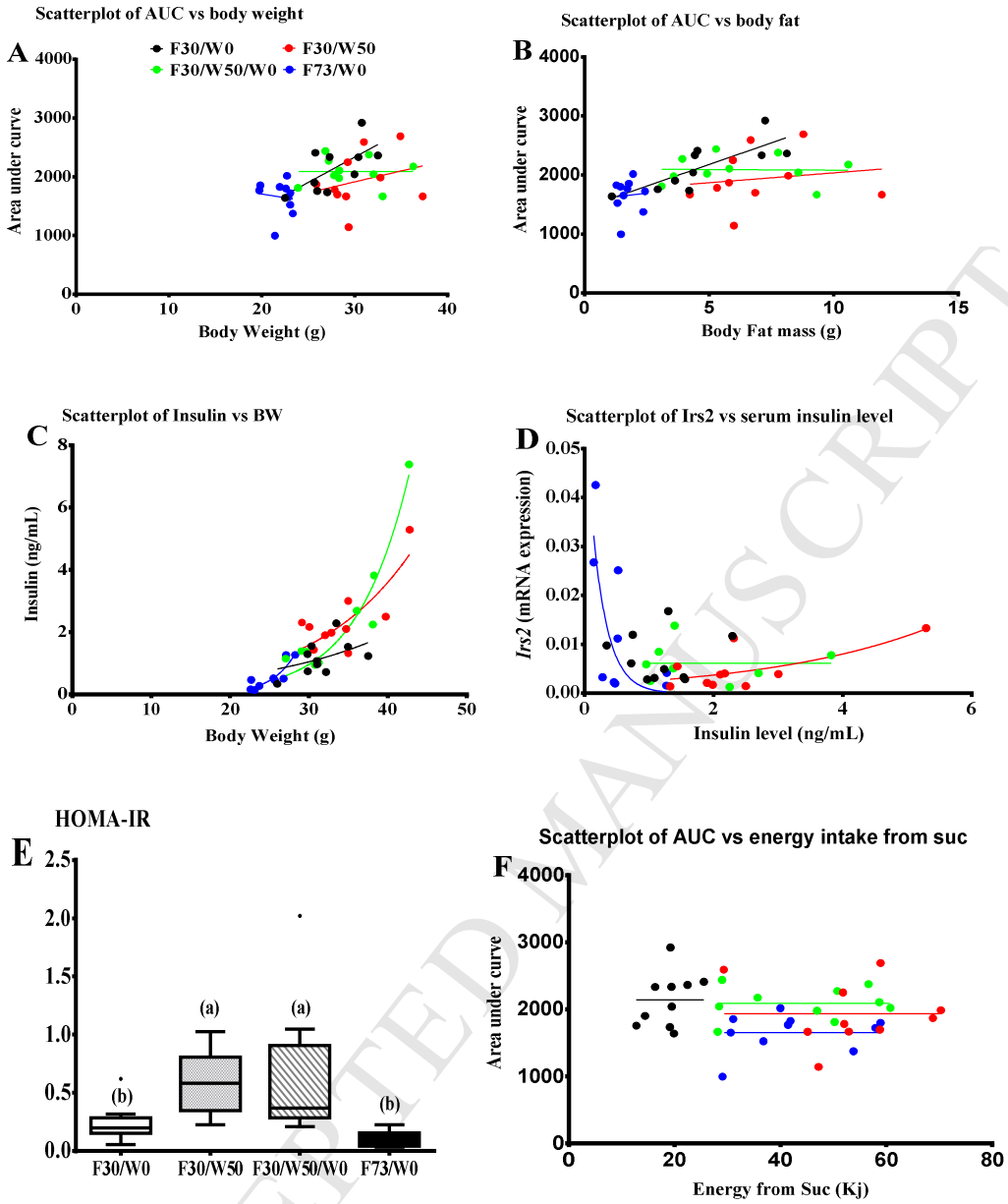

Figure 6

Figure legends

Figure 1: Liquid sucrose intake led to increased caloric intake and body weight gain.

(A) Body weight (2way ANOVA, $F_{3, 2268} = 552.2$, $p < 0.0001$). (B) Body fat (2way ANOVA, $F_{3, 324} = 74.16$, $p < 0.0001$) was greater in liquid sucrose fed animals. (C) Total daily food intake (2way ANOVA, $F_{3, 2232} = 144$, $p < 0.0001$). (D) Liquid sucrose intake (paired t-test, $t = 10.35$, $p < 0.0001$). (G) Absolute daily sucrose intake ($F_{3, 2196} = 1066$ $p < 0.0001$). (E) Energy intake from solid food (2way ANOVA, $F_{3, 1836} = 1456$, $p < 0.0001$). (F) Energy intake from liquid sucrose (paired t-test, $t = 10.54$, $p < 0.0001$). (H) Total energy intake ($F_{3, 2196} = 229.9$, $p < 0.0001$). The first 10 days represent baseline period for A, C, E, and H. Data are shown as means ± SEM (n=10).

Figure 2: Energetic response to sucrose feeding in C57BL6 mice.

(A) Continuous oxygen measurement in the TSE phenotype machine. (B) Scatterplot of daily energy expenditure versus body weight. (C) Locomotion represented as activity (ANOVA, $F_{3, 36} = 0.299$, $p = 0.82$ for day time); (ANOVA, $F_{3, 36} = 0.71$, $p = 0.55$ night time). (D) Energy assimilation efficiency (ANOVA, $F_{3, 24} = 26.54$, $p < 0.0001$). The graph in panel A presents the average of 60 h period for each diet. Grey columns represent darkness period (night). Data are presented means ±SEM.

Figure 3: Hepatic response to sucrose feeding in C57BL6 mice

(A) Liver wet weight measured immediately upon sacrifice was significantly higher in mice drinking liquid sucrose (ANOVA, $F_{3, 34} = 10.11$, $p < 0.0001$). (B) Liver to body weight was determined (ANOVA, $F_{3, 34} = 5.86$, $p = 0.0024$). (C) Glycogen level was not significantly different among groups. (D) *G6pase* mRNA expression (ANOVA, $F_{3, 33} = 0.13$, $p = 0.93$). (E) Hepatic mRNA expression of *Fasn* (ANOVA, $F_{3, 33} = 0.707$, $p = 0.55$). (F) *Pparγ* (ANOVA, $F_{3, 33} = 7.708$, $p = 0.0005$). (G) Represents hepatic fat content (ANOVA, $F_{3, 33} = 13.47$, $p < 0.0001$). (H) Expression of *IL-6* was significantly higher only between treatments F30/W0 and F30/W50. Values are means ± SEM (n = 9-10). Data were analyzed using one-way ANOVA or *t*-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Means that do not share letters are significantly different.

Figure 4: **Liquid sucrose feeding led to impairment in glucose homeostasis.**

(A) Glucose tolerance test performed after a 14 h fast. Blood glucose concentrations are shown at baseline and following an ip glucose load (2 mg/kg). (2-way ANOVA, $F_{3, 180} = 10.59$, $p < 0.0001$). (B) Area under the curve representation of the data (ANOVA, $F_{3, 36} = 3.705$, $p = 0.02$); ns (ANOVA, $F_{2, 27} = 0.81$, $p = 0.4$). (C) Intraperitoneal insulin tolerance test (ITT) (2-way ANOVA, $F_{3, 138} = 26.11$, $p <$

0.0001). (D) Area under curve analysis (ANOVA, $F_{3, 28}$ = 9.38 p = 0.0002). (E) Serum insulin level (ANOVA, $F_{3, 33}$ = 8.70, p = 0.0002). (F) Hepatic expression of the insulin receptor substrate 2 (*Irs2*) (ANOVA, $F_{3.33}$ = 3.209, p = 0.03); ns (ANOVA, $F_{2, 25}$ = 1,382, p = 0.26). Results were analyzed using Two- way ANOVA (Panels A, C); One-way ANOVA (Panels B, D, E and F) with Holm-Sidak's multiple comparison tests. t-test was also used to analyze Panels B, D and F). *p < 0.05, **p < 0.01, ***p < 0.001. (ns = non-significant, p > 0.05). Means that do not share letters are significantly different.

Figure 5: Lingual sweet taste receptor genes expression in mice exposed to liquid and solid sucrose

(A) Lingual mRNA expression of the *Tas1r2* gene (ANOVA, $F_{3, 31}$ = 9.49, p < 0.0001); ns (ANOVA, F2, 23 = 0.85, p = 0.44). (B) Lingual mRNA expression of the *Tas1r3* gene ($F_{3, 31}$ = 3.62, p = 0.0023). Data are presented means ±SEM. Means that do not share letters are significantly different.

Figure 6: Correlation between glucose homeostasis and body composition

Non-linear fitting model was used to find correlation between blood glucose level with body weight (A) (R^2 = 0.191, p = 0.0047) and body fat (B) (R^2 = 0.174, p = 0.0073). Serum insulin level was strongly correlated to body weight (C) (R^2 = 0.771, p < 0.001). (D) Correlation between serum insulin level and hepatic *Irs2* expression (R^2 = 0.374). (E) HOMA-IR presented as median with ranges in a Tukey box plot with outliers represented as dots ($F_{3, 34}$ = 6.175, p = 0.0018). (F) Energy intake from sucrose was not correlated to glucose homeostasis (R^2 = 0.0216, p = 0.39). Means that do not share letters are significantly different.

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AUTHOR'S CONTRIBUTION

J.R.S. conceived and designed the project. J.T. conducted the experiments, contributed to the analysis, and co-wrote the paper with J.R.S. J.R.S and J.T analyzed and interpreted the data. S.H., M.L., and C.N. contributed to data collection and discussion. All authors have critically revised the manuscript for intellectual content and approved its final version.

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Highlights

- Sucrose-sweetened water intake was associated with increased energy consumption and greater body fat gain in C57BL/6 mice
- The same level of sucrose in a solid diet did not lead to higher energy intake or elevated body weight and fatness.
- Elevated adiposity due to sucrose-sweetened water intake was correlated with impairment of glucose homeostasis and insulin resistance.
- Glucose homeostasis and insulin resistance were related to adiposity and the mode but not the level of sucrose intake.

Impact of dietary sucrose on adiposity and glucose homeostasis in C57BL/6J mice depends on mode of ingestion: liquid or solid.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest