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Metabolic and cognitive improvement from switching to saccharin or water following chronic consumption by female rats of 10% sucrose solution

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ABSTRACT

High consumption of sugar-sweetened beverages (SSBs) is a risk factor for weight gain and metabolic disease. Whether this risk is reduced by switching to 'diet' beverages containing low-calorie sweeteners (LCS) is controversial. Two experiments modeled whether a switch from SSB to LCS beverages produced positive outcomes on behavioral and metabolic measures. Both experiments consisted of a Stage 1, in which adult female rats received unrestricted access to 10% sucrose solution in addition to chow and water for 4 (Experiment 1) or 8 weeks (Experiment 2). In Stage 2 rats were switched to either saccharin (*Suc-Sacch*) or water (*Suc-Water*) or remained on 10% sucrose (*Suc-Suc*) for a further 4 (Experiment 1) or 7 weeks (Experiment 2). Experiment 2 contained a fourth group that was maintained on water throughout (*Water-Water*). In both experiments energy intake and weight gain in Stage 2 was reduced for *Suc-Sacch* and *Suc-Water* groups relative to the *Suc-Suc* groups and at cull the *Suc-Suc* groups showed poorer insulin sensitivity and greater g/kg fat than *Suc-Water* and *Suc-Sacch* groups. In Experiment 2 short-term place recognition memory was impaired at the end of Stage 1 but recovered to a similar extent in the *Suc-Water* and *Suc-Sacch* groups; when the latter groups were compared with the *Water-Water* group, recovery was found to be essentially complete. A higher saccharin concentration in Experiment 2 than in Experiment 1 increased absolute amounts of saccharin ingested but intake solution volumes remained low. These results show that switching from sucrose to either water or saccharin produces equivalent improvements on both metabolic and cognitive measures.

1. Introduction

The current worldwide obesity epidemic, accompanied by the sharp increase in related diseases such as Type 2 diabetes (T2D), is strongly associated with increased consumption of sugar-sweetened beverages (SSBs). This holds for developed countries like Australia, for advancing countries such as China and India, and for those less developed such as many Pacific island nations [13]. Although other dietary factors also make an important contribution to obesity, there are strong reasons for focusing on reducing consumption of SSBs.

While there has been intensive study of the effects of adding saccharin to rats' wet food (e.g. [26]), relatively few small animal models have studied the effects of sugar solutions of the ~10% concentration that approximates that used in most SSBs consumed by humans [2,7,28,45]. Using a model of providing rats with 10% sucrose solution in addition to chow and water, we have observed similar metabolic derangements to those produced in humans by an unhealthy diet; namely, impaired insulin function, increased liver and plasma triglycerides, and elevated abdominal adiposity that may or may not be indicated by body weight gain. We have reported these effects in male (e.g. [8,30]) and female rats [15], with effects of sucrose observed even after sucrose access has long ended and replaced with food deprivation [17].

Following other researchers (e.g. [3,43]), we also find sucrose-induced impairments in spatial memory and memory for the location of an object, cognitive tasks that depend on healthy hippocampal functioning (e.g. [14,16]). Recovery from impairment of spatial processing in rodents has been examined in four studies, in which the impairment was produced either by a high fat (HF) diet [18,34] or by a cafeteria diet that included access to a sucrose solution [12,44]; only the latter used the combination of place and object recognition testing employed in the present study and Gomez-Smith et al. [12] failed to find an

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impact of diet on performance in a Barnes maze. It appears that the present study is the first to examine recovery from impaired hippocampal function produced specifically by sucrose.

The replacement of SSBs with either water or drinks sweetened with low calorie sweeteners (LCS) is commonly advised on the rationale that removal of sugars should result in a reduction in total energy intake. As a consequence, weight is lost and metabolic state improved. The scientific basis for such advice – that metabolic health may be improved by the simple removal of sugar drinks or their replacement LCS – is not totally conclusive. A recent meta-analysis of human and animal studies concluded that the use of LCS is effective in reducing total energy intake and body weight [27]. Similar results were found in an earlier meta-analysis [20] and by other reviews [4,19,23,24] with some exceptions [22,36]. However, some rodent studies suggest that consumption of LCS increases short-term energy intake [39–42], accelerates body weight gain (e.g. [10,11,38]) and produces metabolic damage (such as impaired glucose tolerance) under some conditions [35].

The above studies commonly employ a design in which animals are naïve to sweeteners. To our knowledge, the effects of LCS in a SSB withdrawal design have been assessed in only one previous experiment [25]. This contained an initial 8-week stage in which one group of rats was given unrestricted access to an 11% sucrose solution in addition to their normal chow and water, a second group was given a 0.2% saccharin solution to which some aspartame was added, and a final group, the control group, was not given any supplement. During a subsequent 8-week period each of the original three groups was subdivided into two sub-groups, one of which continued as before and the other was switched to the other sweet condition or water. The only reported outcome measure was body weight. No difference was detected between the saccharin solution and water either in terms of initial slower weight gain compared to the sucrose rats or in terms of the reduction in rate of weight gain that occurred when rats were switched from sucrose to either water or saccharin.

The above experiment was described in a brief report that lacks detail and is unclear about several aspects of the method [25]. Also, its conclusion is at odds with a more recent study that examined the effects on weight gain of switching between a caloric sweetener (glucose) and a LCS (saccharin) provided in foods ([37]; Experiment 4). In the first 14-day stage of this study animals were fed a daily 30-g ration of either yoghurt or refried beans that was sweetened with saccharin or glucose; those fed saccharin-sweetened foods gained more weight. In a second stage all rats that were fed yoghurt were now fed beans and vice versa; however, half of the rats remained on the same sweetener and half were switched from glucose to saccharin or from saccharin to glucose. The relevant result in terms of the current experiment is that among the rats fed glucose in Phase 1, those switched to saccharin in Phase 2 gained more weight than those maintained on glucose [37].

The present study tested the effects of a “switch” away from sucrose in the context of our model of SSB consumption. To this end, in Stage 1 we exposed all rats to 10% sucrose solution in addition to chow and water; in Experiment 1 this stage lasted for 4 weeks and in Experiment 2 for 8 weeks. At the end of Stage 1 we assessed short-term memory performance on the object/place task and collected metabolic measures of fasting glucose, plasma insulin and blood triglycerides. In Stage 2, two groups of rats underwent a dietary ‘switch’ in which access to sucrose solution was removed and replaced with water or saccharin solution. These groups were compared with a third group that continued with free access to sucrose solution; in Experiment 1 this stage lasted for 4 weeks and in Experiment 2 it lasted for 7 weeks. We repeated object/place testing at the end of Stage 2 to assess whether groups switched to saccharin or water would improve relative to rats continued on sucrose. Specifically, we predicted that “switched” groups would show improvements on the place task, which is hippocampal-dependent and sensitive to high-sugar or combined high-fat, high-sugar interventions [3,16,43]. By contrast, intact memory on the object recognition task is dependent largely on the perirhinal cortex [21] and therefore we did not expect effects of the switch on performance on this task. The absence of any dietary impact on the object task provides a powerful control against the possibility that impaired performance on the place task reflects some general deficit, such as a decrease in the effect of novelty [3]. At the end of Stage 2 we repeated analyses of fasting glucose, plasma insulin, and blood triglycerides, and assessed body fat at cull.

The design of Experiment 2 differed from that of Experiment 1, not only in terms of maintaining the two stages for longer, but also by including an additional group, one given water throughout this experiment. The point of this was to provide a control to assess whether full recovery could be achieved; that is, would switching from sucrose to water or to saccharin improve metabolic and cognitive status to the extent that these rats no longer differed from ones that had never consumed sucrose at any time?

In summary, the aims of the present study were to test whether the switch from sucrose to either saccharin or water led to better outcomes on body weight, place recognition memory, insulin sensitivity, and fat pad mass, relative to rats maintained on sucrose. A secondary question was whether the effects of switching to water were distinct from switching to saccharin.

2. Experiment 1

2.1. Method

2.1.1. Subjects

Thirty female Sprague-Dawley rats were bred in-house at the School of Psychology, University of Sydney, and were offspring from Control mothers in a separate experiment. In brief, male and female control animals (n = 10 per sex) in that experiment consumed only standard chow with free access to water for approximately 6 weeks prior to mating and then throughout gestation and lactation. Three female offspring from each of the ten litters were used. Rats were 6–7 weeks old at the beginning of the experiment described below, when they were housed individually in open-topped cages (44 × 28 × 29 cm) in a temperature- and humidity-controlled room maintained on a reverse light cycle (lights off 1030–2230). Laboratory chow (Specialty Feed®; 14.2 kJ/g) and tap water were available ad-libitum throughout all experimental procedures.

2.1.2. General procedures

Stage 1 lasted 4 weeks and began the day after rats were re-housed in individual cages. Throughout this stage all rats were given unrestricted access to one bottle of 10% sucrose solution in addition to chow and one bottle of water. Body weight and consumption of chow, water and sucrose were measured every third day. Cage bedding was changed every sixth day. On the last day of Stage 1 (Day 28), sucrose and chow were removed at 2100 h for a 12-h fast corresponding to animals' light/sleep cycle. On the morning of Day 29, fasting glucose was assessed with a standard glucometer (OneTouch Verio©) by removing the tail tip with a sterile scalpel. An additional 50 µl of blood was collected and diluted 1:1 with saline, then centrifuged at 10000RPM for 20-min prior to the extraction of plasma for quantification of insulin using commercially available ELISAs (ALPCO Diagnostics, USA) and triglyceride content (colorimetric assay as described previously in [8]).

Stage 2 lasted a further 4 weeks and began on the same day that blood samples were taken (Day 29). Within each litter group of 3 sisters, one rat was allocated to the *Suc-Suc* group, one to the *Suc-Sacch* group and one to the *Suc-Water* group. During Stage 2 rats had continuing free access to chow and water; however, while unrestricted access to 10% sucrose was maintained for the *Suc-Suc* group, the *Suc-Sacch* group was now provided with one bottle of 0.1% saccharin solution (Saccharin Sodium Salt Hydrate, *Sigma S-1002*) and one bottle of water, while the *Suc-Water* group had access to water only. Body weight and consumption of food and fluids was measured every third day, as in