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Intermittent access to a sucrose solution impairs metabolism in obesity-prone but not obesity-resistant mice

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HIGHLIGHTS

- Liquid sucrose leads to hyperphagia and body weight gain in obesity-prone (OP) mice.
- This hyperphagia induces fat mass gain, fatty liver and insulin resistance in OP mice.
- Liquid sucrose modulates melanocortin and opioid signaling in the brain of OP mice.
- These effects are reversible after 8 weeks of access to water.
- Obesity-resistant mice are protected from sucrose-induced metabolic consequences.

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ABSTRACT

Consumption of sugar-sweetened beverages is associated with overweight and obesity. In this study, we hypothesized that obesity-prone (OP) mice fed a high-fat high-sucrose diet (HFHS) are more sensitive to consumption of sucrose-sweetened water (SSW) than obesity-resistant (OR) mice.

After 3 weeks of ad libitum access to the HFHS diet (7.5 h/day), 180 male mice were classified as either OP (upper quartile of body weight gain, 5.2 ± 0.1 g, n = 45) or OR (lower quartile, 3.2 ± 0.1 g, n = 45). OP and OR mice were subsequently divided into 3 subgroups that had access to HFHS (7.5 h/day) for 16 weeks, supplemented with: i) water (OP/water and OR/water); ii) water and SSW (12.6% w/v), available for 2 h/day randomly when access to HFHS was available and for 5 randomly-chosen days/week (OP/SSW and OR/SSW); or iii) water and SSW for 8 weeks, then only water for 8 weeks (OP/SSW-water and OR/SSW-water).

OR/SSW mice decreased their food intake compared to OR/water mice, while OP/SSW mice exhibited an increase in food and total energy intake compared to OP/water mice. OP/SSW mice also gained more body weight and fat mass than OP/water mice, showed an increase in liver triglycerides and developed insulin resistance. These effects were fully reversed in OP/SSW-water mice. In the gut, OR/SSW mice, but not OP/SSW mice, had an increase GLP-1 and CCK response to a liquid meal compared to mice drinking only water. OP/SSW mice had a decreased expression of melanocortin receptor 4 in the hypothalamus and increased expression of delta opioid receptor in the nucleus accumbens compared to OP/water mice when fasted that could explain the hyperphagia in these mice. When access to the sucrose solution was removed for 8 weeks, OP mice had increased dopaminergic and opioidergic response to a sucrose solution.

Thus, intermittent access to a sucrose solution in mice fed a HFHS diet induces changes in the gut and brain signaling, leading to increased energy intake and adverse metabolic consequences only in mice prone to HFHS-induced obesity.

Abbreviations: AgRP, agouti-related peptide; AUC, area under the curve; BW, body weight; CART, cocaine- and amphetamine-regulated transcript; CB1-R, endocannabinoid receptor 1; CCK, cholecystokinin; CRH, corticotropin-releasing hormone; DA, dopamine; DIO, diet-induced obesity; DOR, delta opioid receptor; DR, dopamine receptor; FFA, free fatty acids; GLP-1, glucagon-like peptide-1; Gox, glucose oxidation; HDL, high-density lipoprotein; HFHS, high-fat high-sucrose; KOR, kappa opioid receptor; Lox, lipid oxidation; MC4R, melanocortin receptor 4; MOR, mu opioid receptor; NAcc, nucleus accumbens; NPY, neuropeptide Y; NS, non-significant; OP mice, obesity-prone mice; OR mice, obesity-resistant mice; OR, opioid-receptor; POMC, proopiomelanocortin; PYY, peptide YY; SPA, spontaneous physical activity; SSBs, sugar-sweetened beverages; SSW, sugar-sweetened water; TG, triglycerides.

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1. Introduction

In recent decades, increased consumption of sugar-sweetened beverages (SSBs) has been associated with obesity and adverse health outcomes in humans [1–3]. These beverages are often consumed as part of a high-energy diet, especially among young people [4,5]. In this obesogenic environment of fat- and sugar-containing high energy density foods, some individuals are more susceptible (obesity-prone) than others (obesity-resistant) to weight gain [6,7].

Similarly, sucrose-sweetened water (SSW) promotes greater energy intake and/or adverse metabolic effects in laboratory animals fed control diets [8–13]. In addition, intermittent access to SSW is more harmful when ingested with a high-fat diet compared to a control diet [14], highlighting the importance of the combination of obesogenic diets and SSBs. Similar to humans, rodents exhibit a large phenotypic diversity in their sensitivity to obesogenic diet [15–20].

In addition to the association between SSBs and the development of obesity, there is growing support for a link between SSBs and the modulation of neural pathways underlying food intake. The consumption of SSBs alters the expression of neuropeptides involved in the homeostatic control of appetite [8,12] and dopaminergic and opioidergic pathways involved in food reward [14,21,22]. These data suggest that the relationship between SSBs and metabolic dysfunction could be the result of altered brain signaling in areas involved in controlling food intake.

We hypothesized that combining fat- and sugar-containing obesogenic diets with SSBs would have greater metabolic consequences in mice already prone to diet-induced obesity (DIO) compared to obesity-resistant mice, and that the observed differences could reflect an altered gut and/or brain signaling. In the present study, we demonstrate that intermittent access to SSW with a sugar concentration similar to soda increases body weight and fat mass gain and leads to insulin resistance and fatty liver only in mice that are already sensitive to a high-fat high-sucrose diet. These metabolic consequences are fully reversed by removal of the SSW, and are accompanied by a decreased expression of the melanocortin 4 receptor in the hypothalamus and increased expression of delta opioid receptor in the nucleus accumbens.

2. Materials and methods

2.1. Animals

180 male C57Bl/6J mice (Harlan Laboratories) aged 5 weeks (19.2 ± 0.4 g) were housed individually in cages with grid floor in a temperature-controlled room (22 ± 1 °C) with a reversed 12:12-hour light–dark cycle (lights off at 09:30). After arrival, mice were habituated to the laboratory conditions for one week with ad libitum access to chow and water. During the experimental period, mice were fed a modified AIN93M high-fat high-sucrose diet (HFHS) (Table 1) that was moistened (70/30 ratio of powder/water) to minimize spillage. To consolidate feeding behavior (and better assess the consequences of adding access to SSW), the HFHS diet was only available for a 7.5 h period beginning at the onset of the dark period (09:30–17:00) throughout the study, as previously published [14]. In a preliminary study, we also controlled that this schedule did not affect BW gain in chow-fed mice and therefore that this 7.5 h time window was long enough to allow a normal caloric intake (unpublished data). Water was available ad libitum with the food throughout the experiment for all mice. This study was approved by the French National Animal Care Committee (number 12/087) and conformed to European legislation on the use of laboratory animals.

2.2. Selection of obesity-resistant (OR) and obesity-prone (OP) mice

OP and OR mice had the same BW (18.7 ± 0.2 g) and fat mass (2.8 ± 0.1 g) at the beginning of the study. Mice were classified as OR or OP based on their gain in body weight (BW) (3.2 g ± 0.1 for OR mice (lower quartile) < 5.2 g ± 0.1 for OP mice (upper quartile), $P < 0.001$) and fat mass (4.2 g ± 0.1 for OR mice (lower quartile) < 5.3 g ± 0.2 for OP mice (upper quartile), $P < 0.001$) during the first 3 weeks of HFHS feeding. OP mice had larger visceral (1.4 g ± 0.1 vs. 0.8 g ± 0.04 for OR, $P < 0.001$) and subcutaneous fat pads (1.4 g ± 0.1 vs. 0.8 g ± 0.04 for OR, $P < 0.001$) compared to OR mice.

Table 1
Composition of the high-fat high-sucrose (HFHS) diet.

Ingredients	g/kg	% energy
Milk protein	170	13.5
Cornstarch	254	40.8
Sucrose	253.7	
Soybean oil	10	45
Lard	215	
Salt mix	35	
Vitamin mix	10	0.8
Cellulose	50	
Choline chloride	2.3	
Energy content, kJ/g	19.6	

2.3. Experimental design

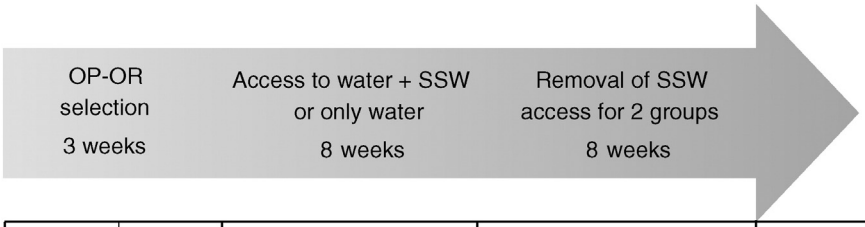
After the 3 week selection period, OR and OP mice were divided into three weight-matched groups (n = 15/group) and continued to receive the HFHS diet for 16 weeks (Fig. 1). Mice had also access to either i) water ad libitum as previously (OR/water, OP/water), ii) water ad libitum plus access to SSW (12.6% w/v sucrose in water, 2.1 kJ/mL) for 2 h/day (at a randomly-chosen time during the 09:00–17:00 time window when access to HFHS was also provided; hereafter referred to as '2 h-intermittent access') on 5 randomly-chosen days/week (OR/SSW, OP/SSW), or iii) water ad libitum plus 2 h-intermittent access to SSW for the first 8 weeks, then access to only water for the last 8 weeks (OR/SSW-water, OP/SSW-water). Access to the SSW was restricted (2 h) and unpredictable (change of time and days of access) to mimic human environment.

2.4. Body weight and body composition

Mice were weighed twice a week. Body fat and lean mass were determined in vivo by dual energy X-ray absorptiometry every 4 weeks (Lunar Piximus, GE Medical System).

2.5. Energy expenditure and food/drink intake

Food and SSW intake were measured twice a week by determining changes in the weight of individual food cups (placed on a grid floor). Data were corrected for spillage by weighing the food that went through the grid, moistening of the solid food and evaporation and converted to kJ. Detailed recordings of HFHS and SSW ingestion patterns, spontaneous physical activity (SPA) and VO_2 and VCO_2 were obtained using individual metabolic cages during weeks 14 and 15, for 2 or 3 consecutive days, during which access to HFHS was available from 09:00 to 17:00 as usual. Day 1 in the metabolic cage was used for habituation. Recordings were taken during day 2 for all groups, and for standardization, SSW was made available between 11:00 and 13:00 for OR/SSW and OP/SSW mice. Recordings were taken during day 3 only for SSW mice to study their feeding and SPA patterns in the absence of access to SSW. Food intake, drink intake and SPA data, initially recorded at 5 s interval, were pooled into 10-min intervals. Food/drink intake data were then analyzed to extract the following parameters: meal numbers, meal size (kJ), meal duration (min), ingestion speed (kJ/min) and inter-meal interval (min) (as described previously [14]). Glucose oxidation (Gox) and lipid oxidation (Lox) profiles were computed in Watts (J/s) from VO_2 and VCO_2 (mL/min) [23].



HFHS diet (n = 180)	OP mice (n = 45)	Water	Water	OP/water (15 mice)
		SSW	SSW	OP/SSW (15 mice)
			Water	OP/SSW-water (15 mice)
	OR mice (n = 45)	Water	Water	OR/water (15 mice)
		SSW	SSW	OR/SSW (15 mice)
			Water	OR/SSW-water (15 mice)

Fig. 1. Experimental design. HFHS, high-fat high-sucrose; OP, obesity-prone; OR, obesity-resistant; SSW, sucrose-sweetened water.

2.6. Oral-glucose tolerance test (OGTT)

During week 8 and week 16, an oral glucose load (2 g/kg) was delivered to each mouse via gavage after an 18 h fast. Blood glucose levels were measured before administration of the glucose load and at 15, 30, 60 and 120 min after administration, using a standard glucometer (LifeScan, One-Touch Vita). Blood samples (30 μL) were taken at each time point, and insulin levels were determined by ELISA (Mercodia Mouse Insulin ELISA).

2.7. Fecal corticosterone metabolites (FCM)

Fecal pellets were collected during weeks 14–15 and stored at −20 °C. Samples were analyzed for immunoreactive fecal corticosterone metabolites using a 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay, as described previously [24]. Samples were run in a single batch.

2.8. Plasma/tissue collection and analysis

At week 17, mice were anesthetized (2.5% isoflurane in 1.2 L/min O_2) and killed by decapitation. All mice were food-deprived overnight. 90 min prior to anesthesia, half of the mice in each group received 0.6 mL (1.3 kJ) of SSW while the other half was kept food-deprived. The main tissues (subcutaneous, retroperitoneal, inguinal and mesenteric fat pads and lean tissue) and organs (liver, spleen, kidneys, lungs, brain and heart) were harvested, blotted dry, and weighed to the nearest 0.001 g. The ileal mucosa was flushed with ice-cold sterile PBS then gently scraped and frozen in Trizol in liquid nitrogen. The brains were removed quickly and the hypothalamus was dissected and snap-frozen in Trizol® reagent. The rest of the brain was frozen in PBS for 2 h at −20 °C. The nucleus accumbens (NAcc) was harvested using a cooled mouse brain matrix (Braintree Scientific, INC) then snap-frozen in Trizol® and stored at −80 °C. Blood was collected in EDTA blood collection tubes containing 10 μL of a protease inhibitor cocktail (cOmpletemini, Roche, Germany). This mixture was centrifuged at 3000 *g* at 4 °C for 10 min to separate the plasma, which was stored in aliquots at −80 °C. The levels of cholesterol, high-density lipoprotein-cholesterol (HDL-cholesterol), free fatty acids (FFAs), triglycerides (TGs) and glucose in the plasma were measured using an Olympus AU 400 automatic chemical analyzer. The TGs were extracted from liver samples using a buffer solution (NaCl, Tris HCl and Triton X100), and the levels were measured using a TG kit (Randox). For hepatic histology, frozen sections of liver embedded in OCT compound were stained with Oil Red O for neutral lipids.

2.9. qPCR analysis

Brain tissues suspended in Trizol® reagent (1 mL) were homogenized using a TissueLyser II (Qiagen) and ileal mucosa was disrupted in Trizol by repeated needle aspiration. RNA concentrations were measured with a NanoDrop ND-1000 UV–Vis spectrophotometer. 0.4 μg of total RNA was reverse transcribed using a high-capacity cDNA archive kit (Applied Biosystems). Real-time PCR was performed using an ABI 7300 Real-time PCT System (Applied Biosystems) and Power SYBR Green PCR Master Mix using the following conditions: 10 min at 95 °C, 40 cycles of 95 °C for 15 s and 1 min at 60 °C [14]. The efficiency was estimated using a series of five-fold dilutions of the sample and confirmed for each run. A melting curve was performed to confirm the absence of contamination. The primer sequences of the target genes are listed in Supplemental Table 1. Gene expression was calculated as $2^{-\Delta Ct}$. Primers specific for the housekeeping genes 18S and RPL13A were used as controls. Gene expression values are expressed relative to RPL13A (similar results were obtained with 18S).

2.10. Statistical analysis

Data are expressed as means ± SEMs. Two-way analysis of variance (ANOVA) was performed to determine the effects of the sensitivity to obesity (OP vs. OR), of the drink (water, SSW-water and SSW) and the interactions between these factors. Time was added as a repeated factor for analysis of body weight gain, fat mass gain, food/drink intake, SPA, glucose oxidation and lipid oxidation. Multiple comparison analysis was performed with Bonferroni post hoc tests (to compare sub-groups within OR and OP mice groups). A P-value below 0.05 was considered to be statistically significant. For the glucose tolerance tests, areas under the curve (AUC) were calculated by trapezoid analysis and compared by one-way ANOVA. All analyses were performed using R 2.15.2.

3. Results

3.1. Body composition

SSW consumption had an overall effect on body weight (BW) gain and body fat mass compared to consumption of water only ($P < 0.001$). This effect was significant only in OP mice, not in OR mice (Fig. 2A and B). In particular, OP/SSW mice had larger epididymal, mesenteric and retroperitoneal fat pads compared to OP/water mice (Table 2). BW and composition of OP/SSW-water mice was similar to OP/water mice, despite the fact that they had a greater BW and fat mass at week 8 (data not shown), indicating that removing access to

SSW reversed the BW and fat mass gain induced by having access to SSW. There was no difference in the final lean mass between the groups and we did not observe any difference in body composition between OR groups.

3.2. SSW and food intake

There was no difference in SSW intake between OR/SSW and OP/SSW mice, as measured manually in the standard cages from week 1 to week 13. When measured in the metabolic cages (during weeks 14 and 15), SSW intake was greater in OP mice than in OR mice (0.7 mL ± 0.1 > 0.5 mL ± 0.05, P = 0.008), due to a non-significant increase in the size and number of drinking bouts. In addition, the first bout of SSW taken by OP mice was larger than that taken by OR mice (0.19 mL ± 0.03 > 0.12 mL ± 0.01, P = 0.04). SSW intake accounted for 3.2% of total energy consumed by OR/SSW mice and 4% of the energy consumed by OP/SSW mice (NS difference = non significant difference, Fig. 2C).

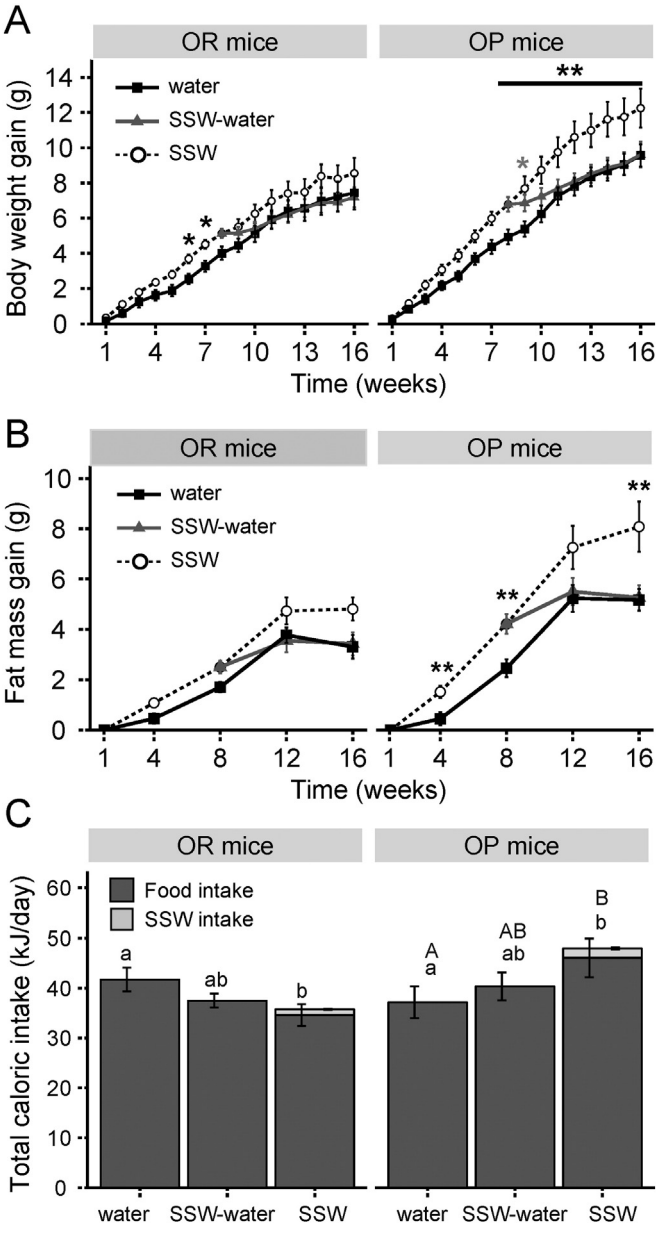


Fig. 2. Effect of SSW access on (A) body weight gain, (B) fat mass gain and (C) daily food and SSW intake (during weeks 14–15) in OR and OP male C57BL/6 mice fed a HFHS diet. Week 1 represents the first week of SSW access (except for water groups), after the 3 weeks selection period of OP/OR. Data are presented as mean ± SEM (n = 15/group). ANOVAS: (A) Repeated measure: OP/OR, drink, time (P < 0.001) and OP/OR × drink interaction (P = 0.003). (B) Repeated measure: OP/OR, drink, time (P < 0.001) and tendency for OP/OR × drink interaction (P = 0.09). (C) Total intake: OP/OR x drink interaction (P = 0.005). A, B: Asterisks indicate significant differences compared to the control (water) groups (*P < 0.05; **P < 0.01). C: Values labeled with different letters are significantly different, P < 0.05. The small letters depict differences for food intake, and the capital letters depict differences in the total energy intake. OR, obesity-resistant; OP, obesity-prone; SSW, sucrose-sweetened water.

On days when OR/SSW mice had access to SSW, they compensated for the SSW intake by decreasing their food intake compared to OR/water mice (P = 0.03, Fig. 2C). This resulted in a tendency for decreased total (food + SSW) energy intake compared to OR/water mice (P = 0.06). In contrast, OP/SSW mice exhibited an increase in their food intake compared to OP/water mice when they had access to SSW (P = 0.02, Fig. 2C), due to a non-significant increase in both meal size and meal frequency (data not shown). Consequently, these mice had a greater total energy intake than OP/water mice (P = 0.006, Fig. 2C), resulting from a greater number of SSW and food meals (19.8 ± 0.8 > 13.6 ± 1.1; P = 0.02).

OR/SSW mice consumed similar amounts of food and total energy when they had access to SSW for 2 h and when they had access to water only (total energy intake: 35.7 kJ ± 2.2 vs. 35.5 kJ ± 1.8, respectively). There was also no significant difference in food intake by OP/SSW mice when they had access to SSW for 2 h and when they had access to water only (46.1 kJ ± 3.5 vs. 41.5 kJ ± 1.8, respectively, NS). However, when SSW was available, the total energy intake of OP/SSW mice tended to be greater than days when only water was provided (47.9 kJ ± 3.9 > 41.5 kJ ± 1.8, respectively, P = 0.09).

There was no difference in food intake between water and SSW-water groups after week 8 in both OR and OP groups (data not shown).

3.3. Spontaneous physical activity (SPA), glucose oxidation and lipid oxidation

OP mice were less active than OR mice (P < 0.001) and having access to SSW reduced SPA levels in OR mice compared to both OR/water mice (P = 0.005) and OR/SSW-water mice (P = 0.01) (Supplemental Fig. 1A). Access to SSW had no effect on SPA in OP mice. Both OR and OP mice were less active during days with access to water only compared to days when they had 2 h of access to SSW (P < 0.01) (Supplemental Fig. 1B). In addition, consumption of the HFHS diet (after 9:30) increased glucose oxidation (Gox) and decreased lipid oxidation (Lox) compared to the food-deprived state (before 9:30) to a similar extent in all groups (data not shown). Having access to SSW between 11:00 and 13:00 did not lead to significant changes in Gox or Lox in OR/SSW and OP/SSW groups (Supplemental Fig. 1C).

3.4. Glucose tolerance, lipid accumulation in the liver, fecal corticosterone metabolites and circulating metabolites

There was no difference in glucose and insulin responses to OGTT between the groups at week 8 (data not shown). At week 16, fasting plasma glucose and insulin did not differ between the groups. All groups of OR mice had similar glucose and insulin response to OGTT (Fig. 3A and B). However, OP/SSW mice had higher blood glucose 15 min after OGTT and higher insulin levels compared to OP/water mice (Fig. 3A and B), as indicated by a larger AUC for both blood glucose (23,269 ± 872 > 20,467 ± 615, P = 0.03) and blood insulin (217 ± 29 > 153 ± 22, P = 0.04).

We observed excessive fat deposition in the form of large lipid droplets, characteristic of macrosteatosis (Fig. 4A), in both OR/SSW and OP/SSW mice. In OP/SSW mice only, this was accompanied by hepatocyte ballooning, suggesting potential liver damage. After removal of SSW, steatosis was reduced in both OR and OP mice and only small droplets could be observed (microsteatosis). Substantial differences were observed in liver triglyceride accumulation depending on the sensitivity

Table 2
Effect of SSW access on body composition in OR and OP male C57BL/6 mice fed a HFHS diet. The data are presented as the mean ± SEM (n = 15/group). Values labeled [a] are significantly different than the corresponding values labeled [b], P < 0.05. OR, obesity-resistant; OP, obesity-prone; SSW, sucrose-sweetened water.

	OR mice			OP mice		
	Water	SSW-water	SSW	Water	SSW-water	SSW
Lean mass gain (g)	2.4 ± 0.4	1.9 ± 0.3	3.3 ± 0.7	2.0 ± 0.4	2.0 ± 0.4	2.7 ± 0.6
Fat mass gain (g)	3.3 ± 0.5	3.4 ± 0.5	4.8 ± 0.5	5.2 ± 0.4[a]	5.3 ± 0.5[a]	8.1 ± 1.0[b]
Final lean mass (g)	20 ± 0.5	19.4 ± 0.3	20 ± 0.5	20.9 ± 0.4	21 ± 0.5	21.9 ± 0.5
Final fat mass (g)	4.3 ± 0.4	4.2 ± 0.3	4.7 ± 0.5	6.2 ± 0.2[a]	6.4 ± 0.3[a]	8.1 ± 0.6[b]
Epididymal fat (g)	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.6 ± 0.1[a]	1.7 ± 0.1[a]	2.3 ± 0.2[b]
Mesenteric fat (g)	0.63 ± 0.04	0.57 ± 0.03	0.64 ± 0.05	0.79 ± 0.03[a]	0.81 ± 0.04[a]	1.03 ± 0.06[b]
Retroperitoneal fat (g)	0.43 ± 0.08	0.43 ± 0.05	0.51 ± 0.06	0.63 ± 0.04[a]	0.73 ± 0.05[a]	0.91 ± 0.06[b]
Subcutaneous fat (g)	2.1 ± 0.2	2.1 ± 0.2	2.3 ± 0.2	3.1 ± 0.1	3.1 ± 0.2	3.8 ± 0.4
Liver mass (g)	0.95 ± 0.04	0.95 ± 0.02	0.93 ± 0.07	1.04 ± 0.04[a]	1.04 ± 0.04[a]	1.19 ± 0.05[b]

to obesity (P < 0.001) and SSW access (P < 0.05). OP/SSW mice had greater mean triglyceride accumulation than OP/water mice (Fig. 4B).

Fecal corticosterone metabolites were also higher in OP/SSW mice compared to OP/water mice (Fig. 5). In the food-deprived state, OP/SSW mice also had higher plasma cholesterol levels than OP/water mice (Supplemental Table 2). Finally, OP mice had greater plasma levels of total and HDL cholesterol compared to OR mice, whether food-deprived or not, and lower levels of hydroxybutyric acid in the plasma after ingesting 0.6 mL of SSW (Supplemental Table 2).

3.5. Expression of GLP-1 and CCK mRNAs in the ileum

Ingesting 0.6 mL of SSW (1.3 kJ) induced greater expression of GLP-1 mRNA in OR/SSW mice (P = 0.047, Fig. 6) and greater expression of CCK mRNA in both OR/SSW and OR/SSW-water mice compared to OR/water mice (P = 0.002 and P = 0.001, respectively). OP/water mice expressed higher levels of CCK mRNA than OR/water mice (P = 0.01), but SSW consumption did not further increase CCK expression in OP mice (Fig. 6).

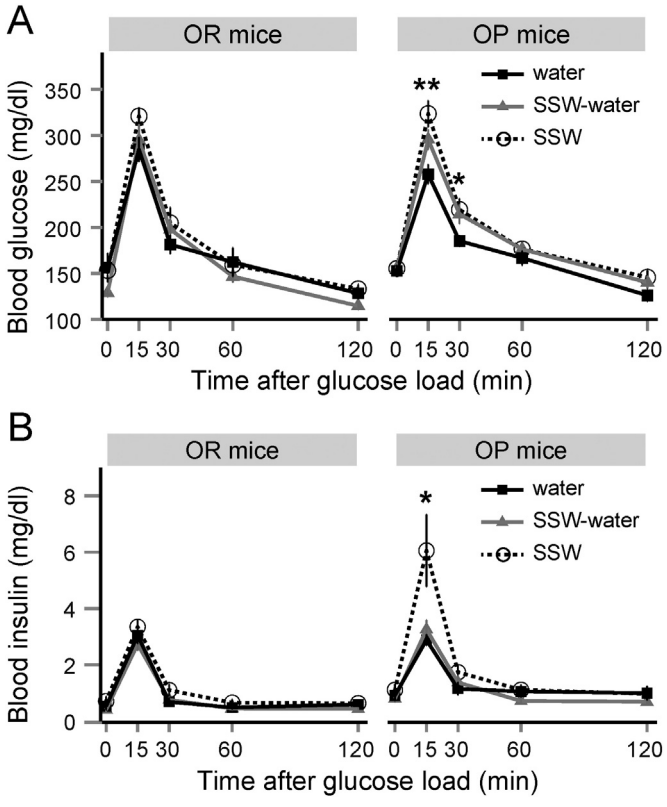


Fig. 3. Effect of SSW access on glucose tolerance in OR and OP male C57BL/6 mice fed a HFHS diet. (A) Blood glucose and (B) insulin were measured before administration of the glucose load (2 g/kg) and 15, 30, 60 and 120 min after administration. Data are presented as mean ± SEM (n = 15/group). * indicates a significant difference compared to the control-water groups (*P < 0.05; **P < 0.01). OR, obesity-resistant; OP, obesity-prone; SSW, sucrose-sweetened water.

3.6. Expression of neuropeptides/receptor systems in the hypothalamus and NAcc

In the hypothalamus of food-deprived mice, OP/SSW mice expressed lower levels of MC4R mRNA than OP/water mice (Fig. 7A) and there was a trend toward decreased POMC mRNA expression in SSW groups compared to water groups (P = 0.09). AgRP expression was lower and NPY expression tended to be lower (P = 0.065) in OP vs. OR mice (Fig. 7A). After ingesting SSW, OP mice tended to express lower levels of MC4R than OR mice (P = 0.051, Fig. 7B).

In the NAcc of food-deprived mice, mRNA expression of delta-opioid receptor (DOR) was higher in OP/SSW mice than in OP/water and OP/SSW-water mice (Fig. 7C). mRNA expression of dopamine receptor 2 (DR2) and DOR were higher in OP vs. OR mice (Fig. 7C). We also observed a trend toward higher expression of the mu-opioid receptor (MOR) (P = 0.08, data not shown). After ingesting SSW, OP/SSW-water mice exhibited higher levels of dopamine receptor 1 (DR1) and opioid receptor delta and kappa (KOR and DOR) mRNA expression compared to OP/water mice (Fig. 7D).

4. Discussion

Consumption of liquid energy is associated with metabolic consequences in rodents. Using groups of mice with different susceptibility to obesity, we showed that intermittent access to SSW in addition to a HFHS diet increases food and total intake only in obesity-prone mice. This increased total energy intake is mirrored by the metabolic data, as OP/SSW mice gained more body weight and liver fat mass, and deposited more epididymal, mesenteric and retroperitoneal fat than OP/water mice. In contrast, OR mice that had intermittent access to SSW reduced their food intake enough to compensate for the energy ingested from the SSW. Previous studies have shown that intermittent access to sucrose solutions [25], vegetable shortening [26] and sweetened condensed milk [27] do not induce a gain in BW after several weeks of consumption. Our results thus show that being OP confers vulnerability to the obesogenic effects of SSW. Importantly, consumption of the HFHS diet induces harmful metabolic effects in most rodents [28], which makes the SSW-induced effects that we observed in OP mice even more remarkable, as OR mice most likely also suffered from some degree of metabolic dysfunction due to the HFHS diet. Access to HFHS was restricted to a defined eating period during this study (as previously published [14]) and access to food was granted immediately at the beginning of the dark cycle to avoid any potential metabolic stress due to restricting food intake when the lights were turned off. We controlled that this food-limitation paradigm didn't induce any change in body weight compared to unlimited access in chow-fed mice in a

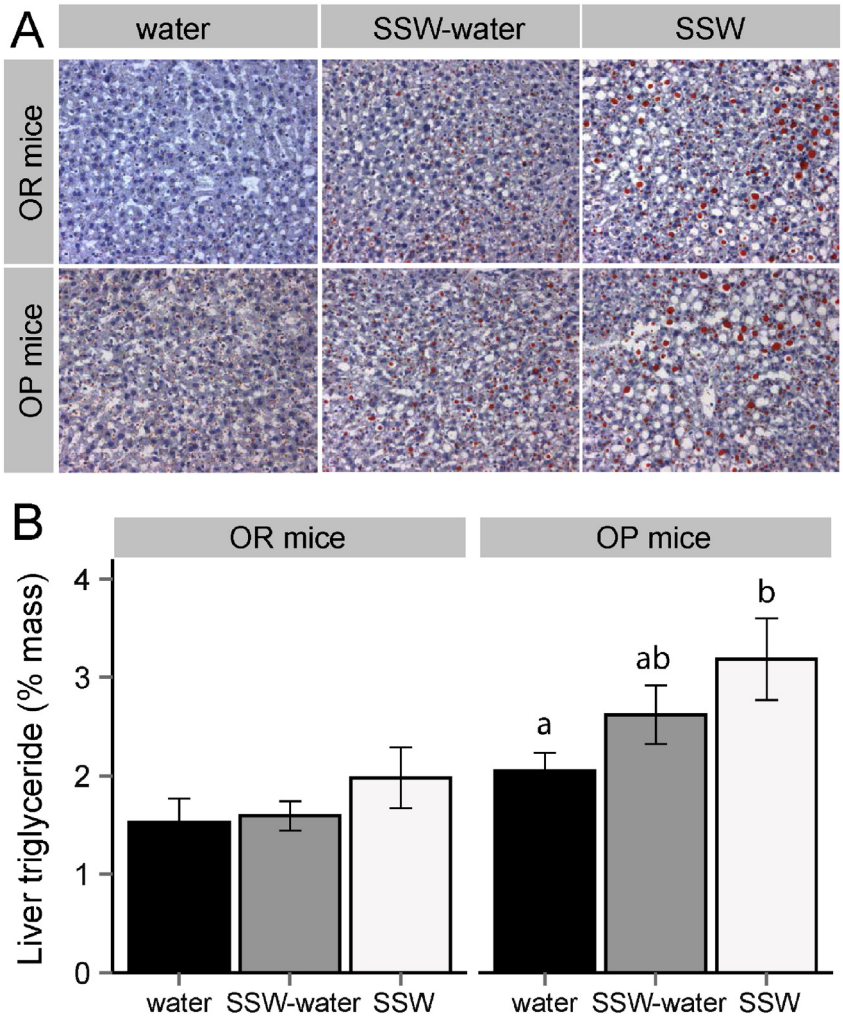


Fig. 4. Effect of SSW on hepatic lipid accumulation in OR and OP male C57BL/6 mice fed a HFHS diet for 16 weeks. (A) Photomicrographs of liver sections stained with hematoxylin-eosin and Oil Red O. The stained sections are from mice with liver triglycerides levels that corresponded to the mean levels from their group. (B) Hepatic triglycerides levels at the end of the study. Data are presented as mean ± SEM (n = 15/group). Values labeled with different letters are significantly different, $P < 0.05$. OR, obesity-resistant; OP, obesity-prone; SSW, sucrose-sweetened water.

previous experiment (unpublished data). However, we cannot exclude that it may have played a role for the SSW-induced obesity phenotype observed in OP mice.

When given intermittent access to SSW, OR/SSW mice decreased their food and total energy intake, while OP/SSW mice ate more food than control OP/water mice, leading to a greater total energy intake. This increased total intake resulted from the combination of increased meal size and meal frequency, both of the HFHS diet and the SSW. This is consistent with a previous study in which rats with ad libitum access to chow, water, lard and a 30% sucrose solution were hyperphagic and ate more meals (both liquid and solid meals) compared to rats fed a solid diet of similar composition [29]. In addition, OP/SSW mice tended to consume more energy on days when they had access to SSW access compared to days when they only had access to water. This could be due to having a choice between two highly palatable foods, leading to hyperphagia.

Brief and intermittent access to a sucrose solution has been shown to induce binge-drinking in rats [30]. However, in the present study, mice with 2 h-intermittent access to SSW did not ingest larger amounts of the solution in the first sip and did not escalate their SSW intake over the 16 weeks of access (based on two criteria used to define bingeing). This observation is in line with previous data using the same experimental design in mice fed a control diet or a high-fat diet [14]. The main difference from other experimental designs is that SSW access was randomized and intermittent, so that mice could not anticipate when they would have access to the sucrose. This led to uncertainty, an important parameter in the control of feeding behaviors [31].

OP/SSW mice exhibited glucose intolerance and released greater amounts of insulin (2-fold increase) compared to OP/water mice, with a mean peak level of plasma glucose greater than 300 mg/dL at 15 min. This observation reflects the close association between adiposity, lipid accumulation in the liver and insulin resistance [32]. The increased fat mass in OP/SSW mice is most likely due to the stimulation of de novo lipogenesis and TG synthesis in the liver as suggested by (i) increased levels in the liver TG, in agreement with a study in which mice were fed a control diet and a 30% sucrose solution for 22 weeks [33], (ii) higher plasma cholesterol, in line with previous reports [12,33] and (iii) increased levels of fecal corticosterone metabolites, a hormone known to have lipogenic effects on its own or in combination with insulin [34]. In OR mice, these metabolic parameters were not affected by access to SSW. Corticosterone measurement in feces is sometimes considered as an indirect parameter of chronic stress [35]. However, in this study, we did not observe any behavioral sign of stress on feeding and activity behavior. In contrast, high levels of corticosterone are quite systematically

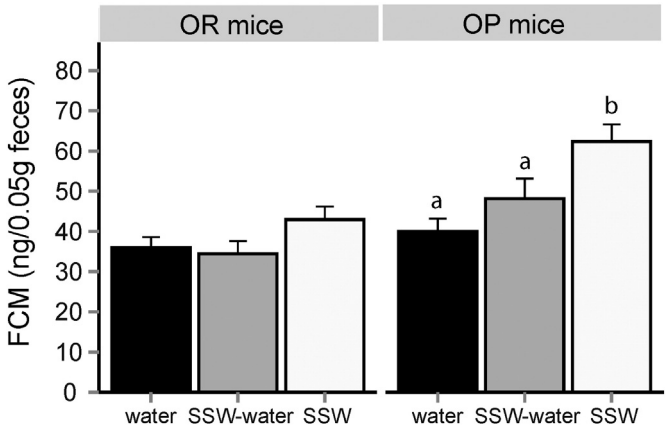


Fig. 5. Effect of SSW on fecal corticosterone metabolites (FCM) in OR and OP male C57BL/6 mice fed a HFHS diet for 16 weeks. Data are presented as mean ± SEM (n = 15/group). Values labeled with different letters are significantly different, P < 0.05. OR, obesity-resistant; OP, obesity-prone; SSW, sucrose-sweetened water.

associated with DIO in mice [36] which suggests that in this study, increased corticosterone level was associated to increased adiposity in OP/SSW mice.

In the present study, the effects of SSW consumption on OP mice were reversible. Indeed, OP/SSW-water gained more weight and fat mass than OP/water mice by week 8, but both groups had a similar body weight and body fat at week 16, 8 weeks after SSW access was removed. In addition, at week 16, OP/SSW-water mice exhibited similar levels of liver TG, plasma cholesterol and a similar response to a glucose load compared to OP/water mice. The reversibility of SSW-induced metabolic effects parallels previous observations that insulin resistance induced by long-term consumption of a HF diet can be reversed by reducing dietary fat [37]. A recent study found that removing ad libitum access to a 10% SSW after 8 weeks in rats fed a control diet led to a decrease in BW over the next 6 weeks, although the rats remained heavier than control rats and had more retroperitoneal fat [38]. Hence, the weight gain induced by ad libitum access to a 10% SSW is less reversible than that induced by 2 h-intermittent access. However, the metabolic profile that OP/SSW-water mice returned to in our study is comparable to that of mice fed a HFHS diet, and this regimen has an established

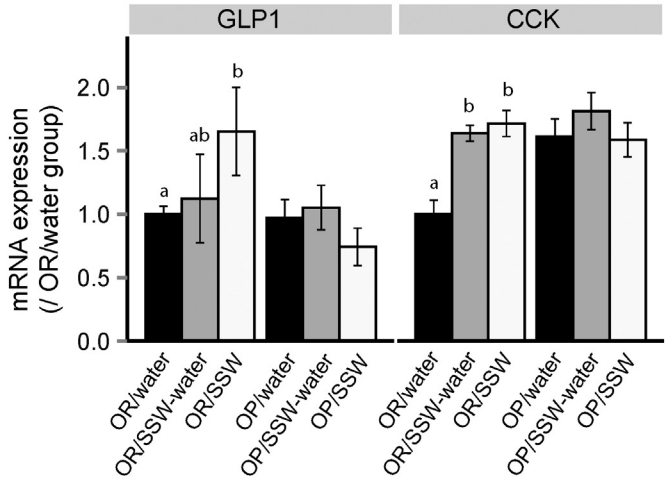


Fig. 6. Effect of long-term SSW consumption on ileal GLP-1 and CCK mRNA expression 90 min after ingestion of SSW (1.3 kJ) in OR and OP mice fed a HFHS diet. Data are presented as mean ± SEM (n = 8/group). Values labeled with different letters are significantly different, P < 0.05. CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; OR, obesity-resistant; OP, obesity-prone; SSW, sucrose-sweetened water.

effect on BW, adiposity and metabolic profile that was not assessed in this study.

Interestingly, acute ingestion of a calibrated sucrose solution (1.3 kJ) resulted in greater ileal GLP-1 mRNA expression in OR/SSW mice vs. OR/water mice, but not in OP/SSW mice vs. OP/water mice. GLP-1 expression increases insulin secretion and insulin sensitivity and reduces food intake [39]. The enhanced anorexigenic response to the sucrose load in OR/SSW mice could indicate an adaptive response that helped mice adjust their energy intake and resist weight gain and the development of insulin resistance when exposed to the HFHS diet and intermittent access to a sucrose solution. Furthermore, expression of CCK, another anorexigenic peptide [39], was increased in response to acute ingestion of SSW in OR/SSW and OR/SSW-water mice compared to OR/water mice but not in OP/SSW and OP/SSW-water mice compared to OP/water mice. As OP/water mice expressed a higher basal level of CCK than OR/water mice, these results could be explained by a ceiling effect on CCK expression induced by the HFHS diet in OP mice.

MC4R expression was down-regulated in the hypothalamus of food-deprived OP/SSW mice (vs. OP/water mice), and POMC expression was also lower in OR/SSW and OP/SSW mice compared to OR/water and OP/water mice. This decreased expression of anorexigenic neuropeptides could be part of a mechanism explaining the hyperphagia observed in OP/SSW mice. This is consistent with data showing a decreased MC4R and POMC expression in the hypothalamus of mice fed a HF diet with 2 h-intermittent access to SSW [14], as well as another study that showed decreased POMC expression in the hypothalamus of rats with access to chow, lard and a sucrose solution [40]. Furthermore, the decreased MC4R expression observed in OP/SSW mice could affect the melanocortin tone in the nucleus accumbens shell through projecting neurons, and affect the motivation for sucrose [41]. Food-deprived OP mice expressed lower levels of AgRP mRNA in the hypothalamus and exhibited a marked, though not statistically significant, decrease in NPY expression compared to OR mice, suggesting a mechanism to help limit energy intake. AgRP and NPY expression did not differ after ingestion of SSW. The feeding status during which the measurements are taken is important, as previous studies have shown that hypothalamic NPY activity is increased in OP vs. OR rats or mice randomly fed [42,43], while other studies observed no difference in AgRP and NPY secretion from hypothalamic explants or hypothalamic mRNA levels between OR and OP mice or rats fed a HF diet [17,44].

Food-deprived OP mice expressed higher levels of dopamine receptor 2 (DR2) and delta opioid receptor (DOR) in the NAcc than food-deprived OR mice. The increased expression of receptors involved in dopamine and opioid signaling observed in OP mice compared to OR mice could be a consequence of greater increases in opioid and DA levels caused by chronic overstimulation from feeding in these mice specifically [45]. We also detected a non-significant increase in mu-opioid receptor (MOR) expression in food-deprived OP mice compared to OR mice, which agrees with the observation that mice with decreased MOR expression in the NAcc are resistant to DIO [46]. In addition, OP mice having consumed sucrose for 16 weeks had increased expression of DOR in the NAcc compared to mice having consumed only water in the food-deprived state. OP mice are hence more susceptible than OR mice to the chronic effect of sucrose on opioid signaling. Remarkably, after 8 weeks withdrawal from sucrose, food-deprived levels of opioid receptors returned to that of OP/water mice. However, SSW ingestion by OP/SSW-water mice induced up-regulation of DR1, KOR and DOR expression compared to OP/water mice, suggesting that re-stimulation with the sucrose solution could lead to over-stimulation of the reward system in OP mice only, even 8 weeks after withdrawal from SSW.

Taken together, our results show that the combination of a HFHS diet and 2 h-intermittent access to SSW for 16 weeks further increases body weight, body fat and fatty liver in mice that are already prone to DIO,

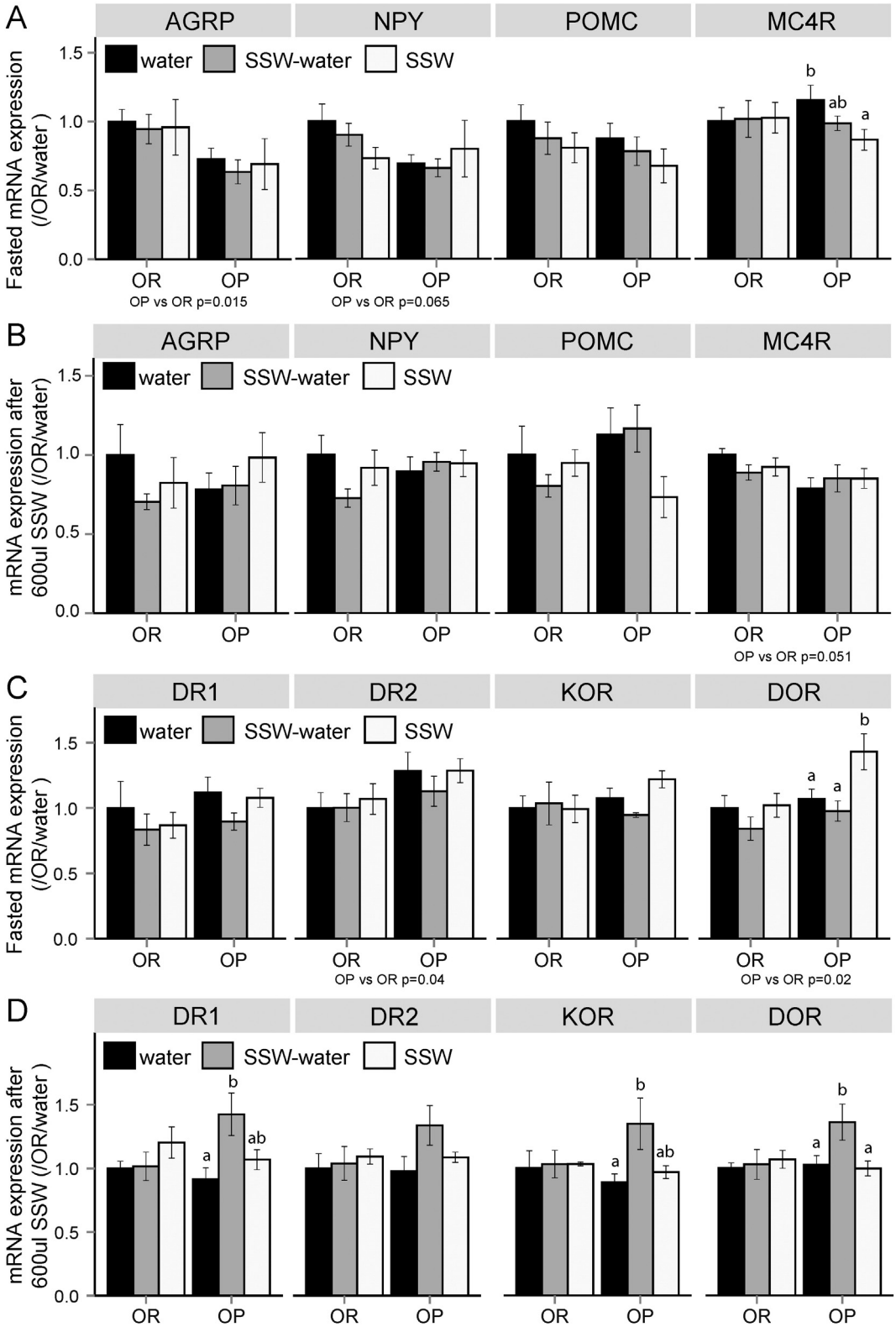


Fig. 7. Effect of long-term SSW consumption on hypothalamic AgRP, NPY, POMC and MC4R (A–B) and DR-1, DR-2, KOR and DOR in the Nacc (C–D) of OR and OP mice. The samples were harvested from mice in a food-deprived state (A–C) or 90 min after ingestion of 600 μL of SSW (1.3 kJ) (B–D). Data are presented as mean ± SEM (n = 7–8/group). Values labeled with different letters are significantly different, $P < 0.05$. DOR, delta opioid receptor; DR, dopamine receptor; KOR, kappa opioid receptor; MC4R, melanocortin 4 receptor; OR, obesity-resistant; OP, obesity-prone; POMC, proopiomelanocortin; SSW, sucrose-sweetened water.

while mice that are resistant to obesity are not affected. A recent study in humans [47] showed an association between genetic predisposition to obesity and SSB consumption and body mass index, but the direct effects of these beverages in obesity-prone or obesity-resistant individuals have not yet been shown. Our results are therefore highly relevant to the current debate over the role of SSBs in obesity and metabolic disorders, as they underscore the importance of individual sensitivity to obesity.

Conflict of interest

All authors state that there is no conflict of interest.

Acknowledgments

M.S., C.C., G.F., D.T. and P.E. designed the research; M.S., C.C., C-D.M. and R.P. conducted the research; M.S. analyzed the data; and M.S., G.F., D.T. and P.E. wrote the manuscript. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2015.11.012.

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