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Behavioral characterization of a model of differential susceptibility to obesity induced by standard and personalized cafeteria diet feeding

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HIGHLIGHTS

- Balb/c mice fed a cafeteria (CAF) diet become obesity prone (OP) or resistant (OR).
- OP and OR mice differ in snack preference (sweet vs savory).
- OP mice decrease their sucrose preference and OR increase their physical activity.
- A personalized CAF diet causes hyperphagia but not obesity in OR mice.

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ABSTRACT

Despite the increase in obesity prevalence over the last decades, humans show large inter-individual variability for susceptibility to diet-induced obesity. Understanding the biological basis of this susceptibility could identify new therapeutic alternatives against obesity. We characterized behavioral changes associated with propensity to obesity induced by cafeteria (CAF) diet consumption in mice. We show that Balb/c mice fed a CAF diet display a large inter-individual variability in susceptibility to diet-induced obesity, such that based on changes in adiposity we can classify mice as obesity prone (OP) or obesity resistant (OR). Both OP and OR were hyperphagic relative to control-fed mice but caloric intake was similar between OP and OR mice. In contrast, OR had a larger increase in locomotor activity following CAF diet compared to OP mice. Obesity resistant and prone mice showed similar intake of sweet snacks, but OR ate more savory snacks than OP mice. Two bottle sucrose preference tests showed that OP decreased their sucrose preference compared to OR mice after CAF diet feeding. Finally, to test the robustness of the OR phenotype in response to further increases in caloric intake, we fed OR mice with a personalized CAF (CAF-P) diet based on individual snack preferences. When fed a CAF-P diet, OR increased their calorie intake compared to OP mice fed the standard CAF diet, but did not reach adiposity levels observed in OP mice. Together, our data show the contribution of hedonic intake, individual snack preference and physical activity to individual susceptibility to obesity in Balb/c mice fed a standard and personalized cafeteria-style diet.

1. Introduction

There are large differences in susceptibility to diet-induced obesity among individuals, but the mechanism(s) governing this variation remains unknown [1,2]. Like humans, rodents exhibit large individual differences in propensity for obesity induced by high-fat diet intake [3–7], and studies suggest that adaptations in energy expenditure and control of hedonic food intake may play a key role [8–10].

High levels of spontaneous physical activity (SPA) correlate with resistance to diet-induced obesity [11–13]. SPA describes low intensity physical activity, executed in the absence of an immediate reward [14]. In humans, SPA is defined as all physical activity excluding formal exercise and describes a series of movements such as ambulating and standing [13,14]. In rodents, SPA includes all locomotor activity in an open field or home cage after an acclimation period to eliminate novelty-induced locomotion [15]. In humans, SPA inversely correlates with weight change during diet-induced obesity whereby people that remain lean spend more time performing SPA compared to those who

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become obese [12,13]. Evidence from animal models also shows SPA can decrease severity of diet-induced obesity. Outbred rats with higher SPA are resistant to obesity induced by high-fat diet compared to rats with low SPA [16] and rats bred for resistance against diet-induced obesity have higher SPA levels compared to rats bred for susceptibility to weight gain when fed high-fat diet [11,17]. Furthermore, SPA was included in statistical models that predict propensity to obesity induced by high-fat diet consumption in inbred C57 mice [4]. However, the relative contribution of SPA to susceptibility to diet-induced obesity in humans and rodents appears to be dependent on the diet type, duration of the over-feeding period and in the case of animal models, of rodent species and/or strain [16,18,19].

In addition to low SPA, excessive intake of energy-dense foods is another major contributor to obesity prevalence [20,21]. Energy-dense foods, such as those rich in fat or sugar alter neuronal circuits regulating reward behavior, thus promoting their over-consumption and facilitating the emergence of obesity [22]. However, there is large inter-individual variability in preference for high-energy dense foods [23,24] that modulates neurobiological adaptations of reward systems and food choice [25]. Furthermore, how obesity alters reward-behavior in relation to food is unclear. In rodents, diet-induced obesity can either increase or decrease food-motivated behaviors such as sucrose pellet self-administration or conditioned place preference for sucrose [26–31]. Animal studies suggest that a higher motivation to obtain energy-dense foods predicts susceptibility to diet-induced obesity [26], which is consistent with neuroimaging studies demonstrating that higher susceptibility to reward effects of energy-dense foods leads to over-eating [32]. Yet, obese rats and humans have lower dopamine tone and release following food intake [33–35], suggesting that over-eating is a compensatory behavior, which seeks to capture the experience of rewards associated with consumption of energy-dense foods [36,37]. Together, these data illustrate that the relative contribution of reward-based behavior to individual susceptibility to obesity remains largely unknown.

Our study sought to determine behavioral adaptations relating to SPA and hedonic food intake in individual susceptibility to diet-induced obesity in mice. We hypothesized that weight change variation among CAF-fed mice would be due to a combination of calorie intake, SPA and hedonic preference. To test this we used Balb/c mice, which have been reported to have a lower susceptibility to diet-induced obesity by high-fat diet consumption compared to other mice strains [3]. Therefore, we fed mice a cafeteria (CAF) diet, which has a higher obesogenic potential compared to homogeneous high-fat diet in pellet form [38,39]. Rodents fed the CAF diet had free access to a rotating selection of energy-dense human snacks plus rodent chow [38,39]. In addition to its higher obesogenic potential, CAF diet is a more translatable model of human unhealthy eating compared to diets rich in a single macronutrient, such as high-fat diets [38,39]. First, we characterized SPA and sucrose preference, before and after CAF diet feeding to determine adaptations of hedonic intake and SPA to individual susceptibility to obesity. Then, we tested whether a personalized CAF diet could induce obesity in mice resistant to diet-induced obesity when fed the CAF diet.

2. Materials and methods

2.1. Animals

Adult male Balb/c mice (8–12 weeks old on arrival, n = 47, Instituto Salud Publica, Santiago, Chile) weighing between 20 and 25 g were housed individually in clear solid bottom cages with corn-cobb bedding and environmental enrichment materials. Mice were maintained on a 12-h light/12-h dark cycle (lights on at 07:00 AM) in a temperature-controlled environment (21–25 °C). For dietary interventions, mice were switched to paper bedding (2:1 mixture of sterilized filter paper and paper towels) to allow precise quantification of food spillage. The control diet was rodent chow (ProLab RMH-3000, Lab Diets, MO, USA, 3.47 kcal/g, with 25.96% kcal from protein, 14.93% kcal from fat and 59.11% kcal from carbohydrates). Supplementary Table 1 shows the nutrient composition of the control diet. Tap water was available *ad libitum* unless noted otherwise. All animal procedures were reviewed and approved by the Institutional Bioethics Committee at Universidad Andres Bello.

2.2. Obesogenic diets

2.2.1. Cafeteria (CAF) diet composition and feeding schedule

The CAF diet contained 6 savory and 6 sweet snacks with an average caloric density of 4.64 ± 0.64 kcal/g and an average macronutrient composition as follows: 8.43% kcal from protein, 45.24% kcal from fat and 46.31% kcal from carbohydrates. Supplementary Table 1 shows the nutrition information for the CAF diet and each snack category. Savory and sweet snacks were classified based on their relative sodium and simple sugar content (Supplementary Table 2).

Mice had continuous access to CAF diet (24-h/d and 7d/week) but snacks were switched 6 d/week every 24 h (Monday–Friday) or 48 h (Saturday to Monday of the following week) with two new randomly selected and pre-weighed sweet and savory snacks. Snacks were placed in a small bowl in a corner of the cage. Mice also had access to standard diet (rodent chow) and tap water *ad libitum* throughout the experiment. To control for changes in food weight due to dehydration, each CAF snack was left at room temperature and weighed daily.

2.2.2. Preferred cafeteria (CAF-P) diet composition and feeding schedule

The CAF-P diet was designed to increase the frequency in which animals were offered their most preferred snacks from CAF diet compared to their less preferred snacks. Following completion of CAF diet feeding, we calculated individual snack preference for each animal by rank ordering based on percent intake throughout the 8 weeks of CAF diet feeding. During CAF-P diet, mice were offered their three most preferred savory and sweet snacks in a ratio of 3:1 relative to their less preferred snacks. The CAF-P diet composition was updated every two weeks in an individual basis, such that the snacks whose consumption represented less than 10% of total snack intake were replaced with the snack that showed the next highest consumption. The CAF-P diet intervention lasted for 6 weeks. All other aspects of the CAF-P dietary intervention were the same as standard CAF diet (Section 2.2.1).

2.2.3. Food intake, body weight and body composition measurements

Food intake from chow and CAF diet snacks (reported as grams (g) or calories (kcal)) were corrected for spillage and dehydration. Body weight (g) was measured every other day. Fat and lean mass (g) were measured weekly (Echo MRI, Houston, TX, USA).

2.3. Spontaneous physical activity (SPA) measurements

Home cage SPA in singly housed animals was recorded continuously using a video camera (SONY CCD 1/3 600 TVL, 15 fps, 352 by 240 pixels) located perpendicular to the longitudinal axis of the cage. To acclimate animals to recording conditions, bedding and enrichment materials were removed 24 h prior to testing, which was necessary for contrast-based detection of the animal. After acclimation, body weight, food intake and spillage were measured. SPA was recorded for 24 h starting 1 h after mice were handled to reduce artificially increasing SPA. Only animals with complete data (i.e. 24 h recordings) were included in the final analysis (OP, n = 16, OR, n = 12, control fed, n = 10). SPA was analyzed with motion tracking software (Any Maze v4.7, Stoelting, IL, USA) and quantified as distance traveled (in arbitrary units) in the horizontal plane based on movement of the animal's center of mass, not including rearing behavior.

2.4. Two-bottle sucrose preference test

The two-bottle sucrose preference test is a widely used protocol to assess individual preference for sucrose [31,40]. This protocol lasted 6 days. On days 1–3, mice were acclimated to the presence of two single spout bottles (45 ml) filled with tap water. On days 4–6, the water in one bottle was replaced with sucrose solution (2.5% *w*/*v*). The position of the bottles within the cage were switched every 24 h to avoid a confounding effect of place preference. Fluid intake was measured every 24 h throughout the experiment and sucrose preference was calculated as percent of sucrose intake relative to total fluid intake. Before CAF feeding, sucrose preference for all animals was measured during two consecutive 24 h periods (days 4–6), and fluid intake data was averaged for analysis. After CAF feeding, sucrose preference for obesity prone (OP) and obesity resistant (OR) mice was measured during a 24 h period both in the presence and absence of CAF snacks. For animals fed the control diet, preference was measured during two 24 h periods and fluid intake data was averaged for analysis. Data from animals that had bottles that leaked (determined by the presence of excessive wet paper bedding) were excluded from the analysis.

2.5. Experimental design

After acclimation to the housing facility, sucrose preference and 24 h SPA were measured in all mice. Next, mice were randomly assigned to either CAF (n = 31) or control (chow, n = 16) diet for 8 weeks. Sucrose preference and 24 h SPA were measured again during weeks 7 and 8. After the 8-week dietary intervention, CAF-fed mice were classified as either OP or OR. Then, a subset of mice (n = 23) was euthanized for tissue collection for an unrelated study. Over the next 8 weeks, the remaining mice OP (n = 8) continued on CAF diet and the control-fed mice remained on chow. The OR (n = 8) mice were fed the personalized diet (CAF-P). Only mice with complete datasets for food intake were analyzed and reported (OP, n = 5; OR, n = 7, CTRL, n = 7).

2.6. Statistical analysis

The effect of CAF diet in OP, OR and control fed mice on body weight, fat and lean mass change (Fig. 1) were analyzed with a two way repeated measures ANOVA in which dietary treatment (CAF vs. control diet; Figs. 1A–C) or group (OP, OR or control-fed; Fig. 1D–F) were the independent variable and time was the repeated measure. The effect of group (OP, OR and control-fed mice) on total caloric intake was analyzed by one-way ANOVA or by repeated measures ANOVA with group (OP, OR or control-fed) as the independent variable and time as the repeated measure (Fig. 2A–C). Differences between OP and OR mice in snack selection (sweet vs. savory) were analyzed with a repeated measures ANOVA or paired t-tests as necessary (Fig. 2D–F). Effects of dietary treatment on sucrose preference (Fig. 3) were analyzed with a paired t-test or a repeated measures ANOVA when necessary. Differences in SPA after dietary intervention (Fig. 4) were analyzed with a two way repeated measures ANOVA with experimental group (OP, OR or control-fed) as the independent variable and dietary intervention as the repeated measure. Differences in intake during CAF-P feeding were analyzed using paired or unpaired t-tests when appropriate. Pairwise analyses were conducted using Student's t-tests corrected for multiple comparisons using either Holm's or false-discovery rate correction. All data is presented as mean ± standard error of the mean (sem).

3. Results

3.1. Effects of CAF diet on body composition and definition of OP and OR phenotype

A total of 47 Balb/c mice were randomly assigned to CAF (n = 32) or control (n = 15) diet for 8 weeks (Fig. 1). There were no significant differences in pre-dietary intervention body weight, fat or lean mass between mice assigned to either diet (Table 1). We also analyzed weekly changes in body weight, fat and lean mass as dependent variables separately using a two way repeated measures ANOVA with diet (control, CAF) as independent variable and time as repeated measure. There were significant effects of time on body weight (Fig. 1A, $F_{8,360} = 93.26$, $p < 0.01$), fat mass (Fig. 1B, $F_{8,360} = 19.79$, $p < 0.01$) and lean mass change (Fig. 1C, $F_{8,360} = 220.17$, $p < 0.01$). For body weight change, we did not find a significant effect of CAF diet ($p = 0.39$) or a significant interaction between dietary intervention and time ($p = 0.77$). However, CAF diet significantly increased fat mass compared to control (Fig. 1B,

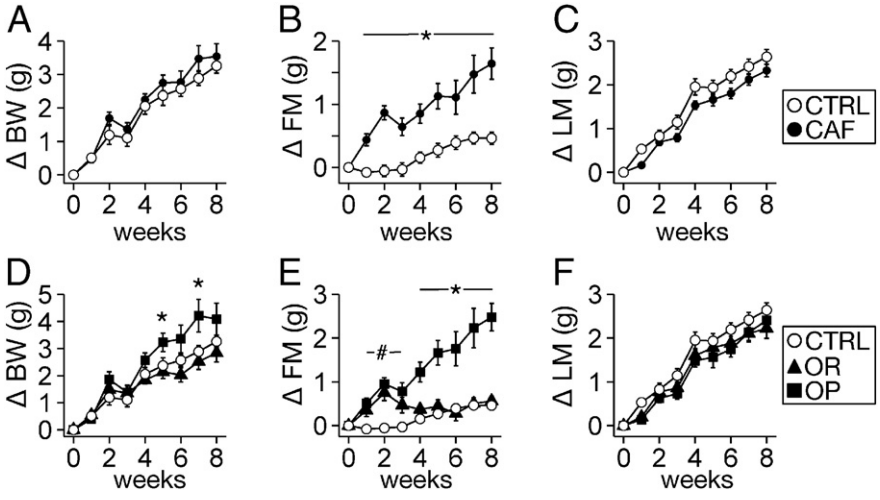

Fig. 1. Effects of CAF diet on body weight and body composition in obesity prone (OP) and obesity resistant (OR) mice. Balb/c mice were fed with CAF (n = 32) or control (n = 15) diet for 8 weeks. Effects of CAF diet on (A) body weight change (ΔBW), (B) fat mass change (ΔFM) and (C) lean mass change (ΔLM). Significant effects of CAF diet on ΔFM and ΔLM were observed (See text for details). * indicates significant pairwise difference between CAF and control diet at each time point. CAF fed mice were classified as OP or OR based on their respective percentile increase in adiposity relative to control fed mice (See methods for details). Panels D–F show differences in (D) ΔBW, (E) ΔFM, **(F)** ΔLM between OP, OR and control-fed mice. * indicates $p < 0.05$ for pairwise comparisons in OP vs. OR or vs control-fed mice; # indicates $p < 0.05$ for pairwise comparisons between OP and OR mice compared to control. Plotted values are mean ± sem.

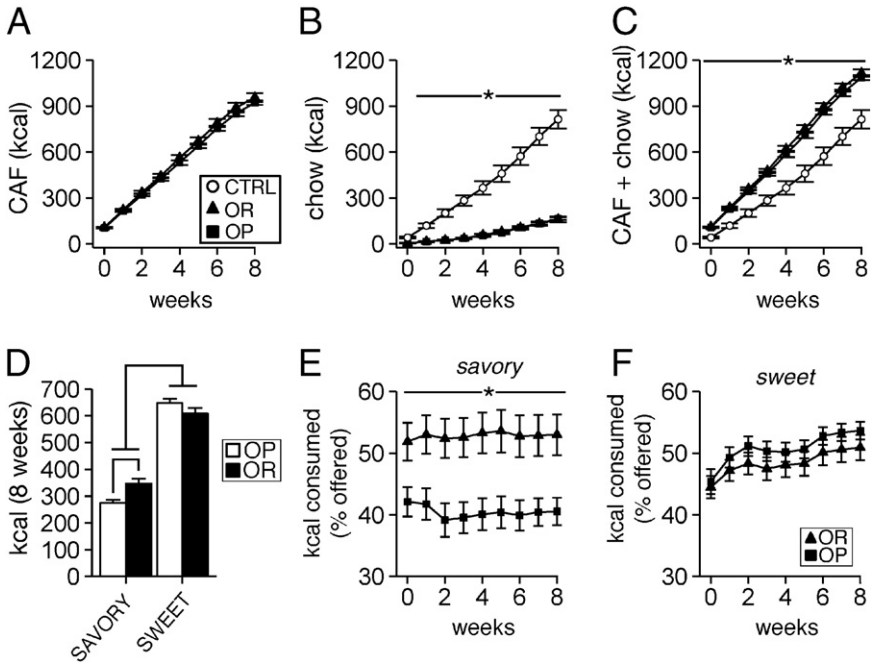

Fig. 2. Food intake and snack preference after CAF feeding in OP and OR mice. Time course of caloric intake of (A) CAF diet, (B) chow and (C) chow + CAF diet (total) in OP, OR and control-fed mice throughout the 8 weeks of dietary intervention. * indicates $p < 0.05$ from pairwise comparisons of OP and OR mice against control. (D) Caloric intake from sweet and savory snack of CAF diet in OP and OR mice. Line indicates $p < 0.05$ for comparisons among phenotypes from pairwise comparisons or main effect of two-way repeated measures ANOVA for comparison between snack types. Panels E-F show time course of consumption of (E) savory and (F) sweet snacks as percent of snack offered. *indicates significant differences between OP and OR mice across each time point from pairwise comparisons. Plotted values are mean ± sem.

$F_{1,45} = 4.13$, $p = 0.001$) with a significant interaction between dietary intervention and time ($F_{8,360} = 2.94$, $p = 0.003$). Finally, there was a small, but significant difference between CAF and control fed mice (Fig. 1C, $F_{1,45} = 4.13$, $p = 0.048$), but CAF and control-fed mice gained lean mass at the same rate (interaction between dietary treatment and time, $p = 0.32$). Pairwise analysis confirmed differences in fat mass change between CAF and control animals (Fig. 1B). Therefore, our data show that CAF diet feeding increases adiposity in Balb/c mice.

Next we examined whether susceptibility to obesity differed within mice fed a CAF diet. First, we determined the variability in the percent increase in adiposity (fat mass relative to lean mass) over the dietary intervention and then classified mice as OP ($n = 18$) if their increase in adiposity was higher than the maximum value of control fed mice at the end of the dietary intervention, and otherwise classified as OR ($n = 14$). Adiposity values were (mean ± sem, range): control diet 0.77 ± 0.45, −1.70 to 4.04; CAF diet, 5.78 ± 0.45, −1.03 to 21.76. Next, we evaluated body weight, fat and lean mass change in OP, OR and control-fed mice using a two-way repeated measures ANOVA with group (OP, OR and control-fed) as independent variable and time as the repeated measure. We observed a significant effect of time on

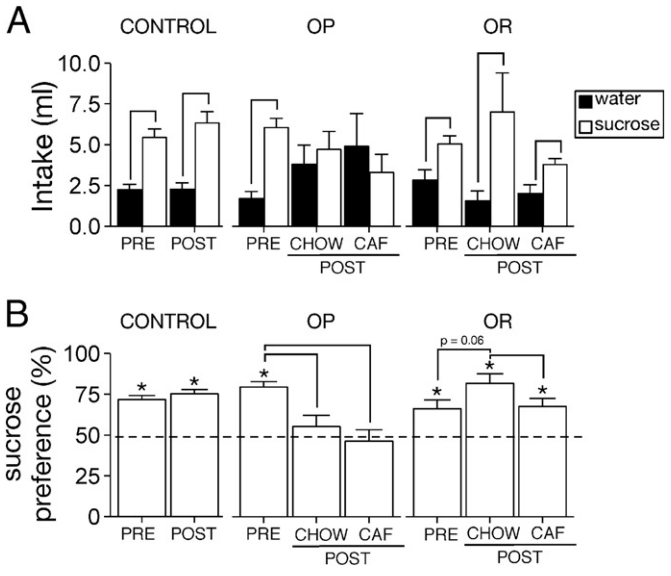

Fig. 3. Sucrose preference in OP and OR mice before and after dietary interventions. For OP and OR mice, sucrose preference post-dietary intervention was measured in presence of standard chow or CAF diet over 24 h. (A) Fluid intake of water and sucrose. Line indicates $p < 0.05$ for pairwise differences between sucrose and water intake. (B) Sucrose preference was calculated as percent of total fluid intake. * indicates $p < 0.05$ for comparison against random preference (50%). Line indicates $p < 0.05$ for pairwise comparisons between all treatments: control, OP or OR mice. Plotted values are mean ± sem.

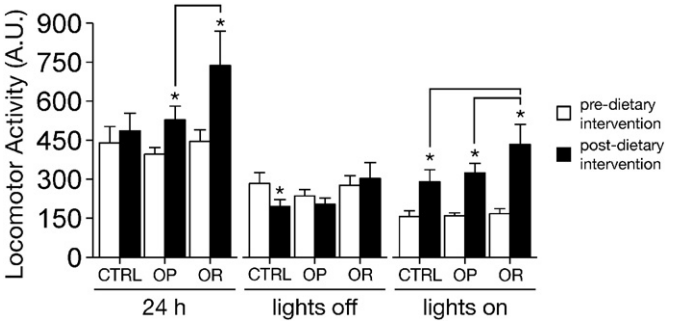

Fig. 4. SPA in OP and OR mice before and after dietary interventions. Spontaneous locomotor activity throughout the 24 h, light and dark periods was measured in OP, OR and control fed animals before and after dietary interventions. Line indicates $p < 0.05$ for pairwise comparisons. *, $p < 0.05$ for pairwise comparison between pre and post dietary intervention within OP, OR and control fed mice. Plotted values are mean ± sem.

Table 1
Baseline characteristics of OP and OR mice and control mice before dietary interventions.

	Body weight[a]	Fat mass[a]	Lean mass[a]
Control diet	25.80 ± 0.31	2.21 ± 0.06	21.29 ± 0.22
CAF	25.81 ± 0.25	2.30 ± 0.06	21.21 ± 0.21
OP	26.14 ± 0.34	2.35 ± 0.09	21.40 ± 0.28
OR	25.39 ± 0.35	2.24 ± 0.09	20.95 ± 0.32

[a] There were no significant differences between diets (t-test) or between OP, OR and control-fed mice (one-way ANOVA), $p > 0.05$ for all analysis.

body weight (Fig. 1D, $F_{8,352} = 102.05$, $p < 0.01$), fat mass (Fig. 1E, $F_{8,352} = 218.78$, $p < 0.01$) and lean mass change (Fig. 1F, $F_{8,352} = 25.42$, $p < 0.01$). There was no main effect of group (OP, OR and control-fed) on body weight change (Fig. 1D, $p = 0.086$) but a significant interaction between group and time (Fig. 1D, $F_{8,352} = 2.95$, $p < 0.001$). Therefore, we examined differences across groups at each time point and observed OP gained significantly more weight compared to OR and control-fed mice after the fifth week of dietary intervention (Fig. 1D). For fat mass change, we observed a significant main effect of group (Fig. 1E, $F_{2,44} = 16.26$, $p < 0.001$) and a significant interaction between group and time ($F_{8,352} = 8.78$, $p < 0.001$). Post-hoc analysis showed OP started to diverge from OR mice in fat mass gain after the fourth week of dietary intervention (Fig. 1E). Finally, we did not find a significant main effect of group on lean mass change (Fig. 1F, $p = 0.13$) or an interaction between group and time ($p = 0.53$). Our data show that mice fed a CAF diet displayed large individual variability in their susceptibility to diet-induced obesity as indicated by adiposity but not body weight.

3.2. Food intake and snack preference in OP and OR mice

Caloric intake for CAF, chow and total (chow + CAF diet) was analyzed separately over the 8 weeks of dietary intervention for OP, OR and control fed mice with a two way repeated measures ANOVA with group (OP, OR, control fed) as independent variable and time as repeated measure. These analyses showed significant effects of time for all dependent variables (Fig. 2A-C, $p < 0.05$ for all variables) and significant differences among groups for chow intake (Fig. 2B, $F_{2,41} = 63.46$, $p < 0.001$) and total intake (Fig. 2C, $F_{2,41} = 22.1$, $p < 0.001$). Paired comparisons showed no significant differences in caloric intake from CAF diet or chow between OP and OR mice (Fig. 2A-B) and both OP and OR consumed significantly less chow compared to control-fed mice (Fig. 2B). Total caloric intake (chow + CAF) the end of the dietary intervention was not significantly different between OP and OR mice. However, both groups had significantly higher caloric intake than control-fed animals (Fig. 2C, one way ANOVA for total caloric intake, $F_{2,41} = 9.26$, $p < 0.01$). Next, we examined macronutrient intake in OP and OR mice receiving the CAF diet. There were no significant differences in macronutrient intake between OP and OR mice, but both OP and OR mice had higher fat and lower protein intake compared to mice fed the control diet (Table 2). Carbohydrate intake in OP and OR rats was the same as that in control-fed mice, but OP and OR consumed fewer complex carbohydrates compared to control-fed mice (Table 2). These data indicate that OP and OR were hyperphagic compared to control-fed mice due to elevated intake of fat and simple sugars.

Next, we examined the proportion of sweet and savory snack intake in OP and OR mice using a one-way ANOVA with group (OP vs. OR) as the independent variable and caloric intake from each snack category as the dependent variable (Fig. 2D). There was no difference in total caloric intake between OP and OR (main effect of phenotype, $p = 0.29$). Both OP and OR mice consumed significantly more calories from sweet compared to savory snacks (main effect of snack category, $F_{1,27} = 31.8.1$, $p < 0.001$) and there was a significant interaction between OP/OR classification and snack category ($F_{1,27} = 9.54$, $p = 0.004$). Pairwise analysis showed OR consumed more calories from savory snacks compared to OP mice (Fig. 2D), which suggests that OR mice prefer savory snacks. To further explore this, we examined the time course of preference for sweet and savory snack intake using a repeated measures ANOVA with group (OP vs. OR) and time (weeks) as the independent variables, and caloric intake from savory and sweet foods as the dependent variables. For savory snacks (Fig. 2E), we observed a significant difference between OP and OR mice ($F_{1,27} = 9.87$, $p = 0.004$), with no significant effect of time ($p = 0.48$) or group by time interaction ($p = 0.31$), on calories form savory foods. In contrast, for sweet snacks (Fig. 2F), there were no significant differences between OP and mice ($p = 0.33$), but there was a significant effect of time ($F_{8,216} = 40.4$, $p < 0.01$) and no significant interaction ($p = 0.64$). Our data show that OR had a higher intake from savory snacks compared to OP mice while intake from sweet snacks was similar in OP and OR mice.

3.3. Sucrose preference in OP, OR and control-fed mice

Sucrose preference was measured with the two-bottle preference test in OP, OR and control-fed mice before and after dietary interventions. Before dietary interventions, all mice were tested in presence of chow. After CAF feeding, OP and OR mice were tested in presence and absence of CAF diet. Before dietary interventions, all groups drank significantly more sucrose compared to water (Fig. 3A), but after dietary interventions, only the control-fed and OR mice (regardless of diet offered during testing) drank more sucrose compared to water (Fig. 3A). Next we calculated sucrose preference as percent of total fluid intake before and after dietary interventions. There was no change in sucrose preference in control-fed mice over time (Fig. 3B, paired t-test, $p > 0.05$). Changes in sucrose preference in OP and OR mice were analyzed separately with a repeated measures ANOVA with time (pre-CAF, post-CAF with CAF diet or post-CAF with chow diet) as the repeated measure and sucrose preference as the dependent variable. For OP mice, there were significant changes in sucrose preference over time ($F_{2,18} = 9.25$, $p = 0.001$) and post-hoc analysis showed a decrease in sucrose preference after CAF feeding such that sucrose preference was not different from chance preference irrespective of the presence of CAF or chow diet during the test (Fig. 3B). For OR mice, there were also significant changes in sucrose preference over time ($F_{2,16} = 4.34$, $p = 0.031$) with an increase in sucrose preference after CAF feeding when exposed to chow diet compared to CAF diet (Fig. 3B) and a difference compared to pre-CAF feeding that failed to reach statistical significance. In conclusion, our data show that the exposure to CAF diet decreases sucrose preference in OP mice when tested with a single sucrose concentration (of 2.5%), and a trend towards an increase in sucrose preference for OR mice.

3.4. SPA in OP, OR and control-fed mice before and after dietary intervention

SPA in OP, OR and control-fed mice was measured for 24 h before and after dietary interventions (Fig. 4) and analyzed by a two-way repeated measures ANOVA with group (OP, OR and control-fed) and

Table 2
Intake of macronutrients, salt and simple sugars for OP, OR mice and control mice after an 8-week dietary intervention.

	Protein (kcal)	Fat (kcal)	Carbohydrates (kcal)	Simple sugars (g)[a]
Control diet	250.36 ± 5.85	143.91 ± 3.36	569.91 ± 13.31	3.07 ± 0.07
OP	110.11 ± 4.99***	440.72 ± 8.37***	539.18 ± 11.46	58.11 ± 1.44***
OR	106.81 ± 5.48***	456.68 ± 11.76***	534.41 ± 16.02	55.25 ± 1.87***

*** $p < 0.01$ when compared to control with pairwise comparison test corrected for multiple comparisons.
[a] For the control diet, simple sugars include sucrose, fructose and lactose while for the CAF snacks, the information from the nutritional label was used.

time relative to dietary intervention (pre- or post) as the independent variables, and SPA as the dependent variable. We found a significant effect of dietary intervention on SPA ($F_{1,35} = 8.22$, $p = 0.007$), but no significant effect of phenotype ($p = 0.21$) or interaction between dietary intervention and group ($p = 0.07$). Pairwise analysis showed no changes in SPA in control-fed animals (Fig. 4). There was a significant increase in SPA after CAF-feeding in both OP and OR mice (Fig. 4), but the SPA levels after CAF feeding were significantly higher in OR compared to OP mice. Analysis of SPA separated by light and dark periods showed that SPA was significantly increased in OR mice in the light period (Fig. 4). In fact, all groups increased their SPA during this period after completion of the dietary intervention. However, the increase in SPA at 24 h and light period was larger in OR mice (Fig. 4). Overall, our data suggest increased physical activity in OR mice after CAF feeding compared to control and OP mice.

3.5. Effects of personalized CAF diet (CAF-P) on obesity development on OR mice

To determine the robustness of the OR phenotype we fed OR mice with a CAF diet personalized for individual snack preferences (CAF-P) for an additional 6 weeks after the 8 weeks of standard CAF diet. The CAF-P diet consisted of mice receiving their three most preferred sweet and savory snacks in a ratio of 3:1 relative to their less-preferred snacks (see the Materials and methods section). The snack composition of the CAF-P diet was re-assessed for individual preferences every two weeks. OR mice had significantly higher weekly caloric intake when fed the CAF-P diet compared to the standard CAF diet (Fig. 5A). OP mice also increased their average weekly intake during this period (weeks 9–14) compared to the first 8 weeks (Fig. 5A). However, OR mice fed the CAF-P diet had higher intake compared to OP mice over the same period (weeks 9–14). Analysis of food intake corrected by body weight (Fig. 5B) eliminated the increase in food intake in OP mice during the 9–14 week period compared to the prior 8 weeks, but did not eliminate the increase in caloric intake in OR mice fed a CAF-P diet compared to CAF diet and OP mice (Fig. 5B). Therefore, the CAF-P diet can potentiate the increase in food intake caused by CAF diet in OR mice.

Next, we evaluated whether increased caloric intake altered body composition in OR mice fed the CAF-P diet. Fig. 5C shows the adiposity increase in OP, OR and control (chow-fed) mice at the beginning of the dietary intervention, week 8 (end of CAF diet) and week 14 (end of CAF-P diet). Despite OR mice increasing their intake compared to OP mice due to CAF-P exposure (Fig. 5A–B), the adiposity levels of were not different of those of control fed mice (Fig. 5C), while OP mice continued to increase their adiposity. Together, these data shows that despite increased intake compared to OP mice due to the CAF-P diet, the OR mice remain resistant to diet-induced obesity.

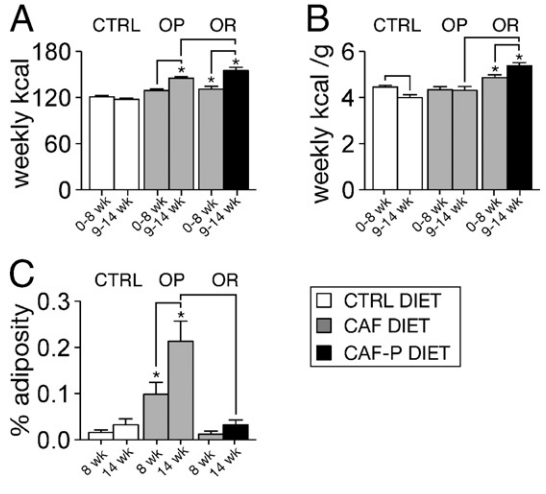

Fig. 5. Personalized CAF diet does not increase adiposity in OR mice. Obesity resistant (OR, n = 7) and OP (n = 5) mice were fed CAF diet for 8 weeks followed by 6 weeks of a CAF diet personalized for individual snack preferences (CAF-P diet) for OR and standard, non-personalized CAF diet for OP mice. CTRL mice (n = 7) were fed a control diet (A) Weekly caloric intake (B) Weekly caloric intake corrected by body weight (C) Change in percent of adiposity. Line indicates $p < 0.05$ for pairwise comparisons, * indicates significant difference from control fed animals for each time period. Plotted values are mean ± sem.

4. Discussion

Here we characterized behavioral mechanisms mediating individual susceptibility to diet-induced obesity in Balb/c mice after CAF diet feeding. The Balb/c strain is resistant to obesity induced by high-fat diet compared to other mouse strains, such as C57Bl/6 [3,6,7]. However, there is variability in complex phenotypes across breeders [15] and thus whether the same differential susceptibility to obesity between Balb/c and C57Bl/6 mice from the breeder used in these studies parallels previous studies remains to be tested. In our studies, we observed a large variability in adiposity change among Balb/c mice fed the CAF diet (Fig. 1), thereby classifying mice into two sub-groups: OP and OR.

Despite lower intake of calories from protein compared to control-fed animals (Table 2), mice fed the CAF diet gained weight (Fig. 1D) and lean mass change was not significantly different between OP, OR and control-diet fed mice (Fig. 1F). Together with the lack of differences in caloric intake between OP and OR mice (Fig. 2) and higher SPA in OR mice compared to OP mice after the CAF diet (Fig. 4), these data supports the concept that increases in SPA as a response to excess energy intake contributes to resistance to diet induced obesity as there were no differences in SPA between OP and OR mice before CAF diet feeding [11,41]. Increases in SPA during the light period after dietary intervention were observed in all mice, suggesting this could be due to daily manipulation in the early AM period for measurement of food intake, body weight and composition (See the Materials and methods section). In conclusion, our data suggest that increased SPA in response to CAF diet feeding contributes to the OR phenotype, but it remains critical to measure overall energy expenditure in OP and OR mice to determine whether the observed changes in SPA are reflected in resting and activity-related energy expenditure.

Obesity prone and resistant mice showed higher consumption of calories from fat, lower from protein and no difference in total calories from carbohydrates compared to control-fed mice (Table 2). However, OP and OR mice had higher consumption of simple sugars, which is consistent with the nutrient content of the CAF diet (Supplementary Table 1). The present results showed no significant differences in total caloric intake between OP and OR mice. Similarly, OP and OR Sprague–Dawley rats fed a high-fat diet had no differences in total caloric intake [41], and food intake was not correlated with weight change across different mouse strains fed a high fat and sucrose diet [6]. However, in rats selectively bred for susceptibility to diet-induced obesity, OP rats had higher intake and energy efficiency when fed energy-dense diets compared to OR rats [42]. Likewise in C57Bl6/J mice, there was a positive linear correlation between weight and fat change with caloric intake [5]. These data illustrate that adaption to energy dense nutrients differs between strains and species of rodent models of obesity. Furthermore, in contrast to other studies examining intra-strain variation in response to long-term high-fat diet feeding in other mouse strains [4,5], our data from Balb/c mice suggest body weight, fat or lean mass prior to CAF feeding does not predict OP/OR status or increased adiposity. These data further suggest that emergence of the OP/OR phenotype is due to a response to CAF feeding rather than baseline adiposity characteristics of mice.

In our formulation of the CAF diet, snacks were classified as either sweet or savory based on their sugar and sodium content, with savory snacks having higher sodium content compared to sweet snacks (Supplementary Table 2). During CAF diet feeding both OP and OR mice had higher preference for sweet compared to savory snacks (Fig. 2)

and OR showed higher intake of savory snacks from the beginning of the CAF diet compared to OP mice. Both OR and OP mice had similar preference for sweet snacks (Fig. 2). The contribution of this difference to preference between OP and OR mice for savory snacks to their susceptibility to obesity is unclear, as there were no differences in total caloric intake between phenotypes. First, although we classified snacks as sweet or savory we did not explore in detail whether OR mice prefer certain snacks based on sodium content or another feature of these snacks, such as texture or added flavors. This aspect of the model deserves further exploration. Secondly, evidence from animal models and humans suggest differential effects of macronutrient preference and pattern of intake in development of obesity and compulsive eating [43–45]. We attempted to use individual snack preference in the CAF-P model to further increase caloric intake and obesity in OR mice, but whether the observed differences in snack preference could be used to develop a model of binge eating based on individual preferences remains an open question. Finally, the opioid peptides have been implicated in the neuronal regulation of macronutrient preferences [25,46] and thus we could hypothesize opioid signaling is critical for expression of snack preference in OR and OP mice. In summary, the difference in snack preference between OP and OR mice suggested it was necessary to study in more detail the adaptations in hedonic intake in OP and OR mice.

To better understand the adaptations in hedonic intake caused by CAF diet feeding, we examined sucrose preference using the two-bottle test in all mice before and after CAF feeding. Sucrose preference decreased in OP mice after CAF feeding irrespective of whether animals had access to CAF diet during testing (Fig. 3). Also, OR mice increased their sucrose preference after CAF feeding but only in the presence of chow (Fig. 3) and it was not different from pre-dietary intervention when tested in presence of CAF diet. The data from OP mice is consistent with the idea that obesity decreases sensitivity to sweet taste, which has been observed in other rodent models of obesity [26,29,30,34,36,37]. However, a definitive confirmation of decreased sucrose sensitivity in OP would require testing sucrose preference of OP and OR mice over a range of sucrose concentrations. For example, it is also possible that OP mice might show increased sucrose preference at higher sucrose concentrations. Future experiments will address this possibility.

A recent study tested sucrose preference in selectively bred OP and OR rats and showed that high-fat diet intake reduced sucrose preference relative to rats fed a control diet [31]. However, OP and OR mice consumed the same total amount of simple sugars during CAF feeding (Table 2), suggesting that for OP mice, it is the obese state, and not the dietary exposure that blunted sucrose preference. The difference in savory snack intake in OP compared to OR mice suggest possible differences in salt preference, however this issue remains to be further studied. Differences in salt sensitivity have been observed in obese subjects [47–49], but evidence is not conclusive regarding whether a difference in salt preference is a risk factor for obesity. Further experiments are necessary to determine the mechanisms behind differential adaptation of sucrose preference and putative differences in salt preference observed in OP and OR mice fed a CAF diet.

Obesity resistant mice had a significantly greater increase in SPA compared to OP mice in response to a CAF diet. Together with the food intake and preference data shown in Figs. 2-3, the results suggest that differences in obesity susceptibility between OP and OR mice (as indicated by adiposity index) are due to higher SPA levels in OR mice in response to CAF diet feeding rather than changes in energy intake (Fig. 4). This has been shown by other studies [4,17,41,50–52], which suggested that differential adaptations in orexin function could explain the OP/OR phenotype. However, a confirmation of the role of SPA and orexins in our OP/OR phenotype requires a direct measurement of energy expenditure associated to physical activity and measurements of orexin-induced SPA.

Our behavioral data show that the OR phenotype is characterized by higher physical activity while the OP phenotype is characterized by decreased sucrose preference. Considering the observed variability in snack preference between OP and OR mice (Fig. 2) and also at an individual level within OR mice, we developed a feeding protocol referred to as personalized CAF (CAF-P) diet to further increase caloric intake in OR mice and to test whether this would be sufficient to drive their body composition towards an OP phenotype. Obesity resistant mice consumed more calories when fed the CAF-P diet compared to OP mice fed CAF diet and when OR mice were fed CAF diet (Fig. 5A-B), but the CAF-P diet failed to increase adiposity in OR mice (Fig. 5C). This finding suggests that the OR phenotype mice in Balb/c mice is resistant to further elevations in caloric intake. Increased energy expenditure and locomotor activity during as CAF intake is the most plausible mechanism contributing to maintaining the OR phenotype (Fig. 5). However, future experiments are needed to explore this possibility.

Originally, we hypothesized that OR mice fed a CAF-P diet would transition into an OP phenotype, and hence we designed our experiments to answer this specific question. Future experiments shall include a fully crossed design between phenotype (OP vs OR) and CAF diet (personalized vs. non personalized) to determine whether OP/OR phenotypes respond equally to a CAF-P diet. Although there are dietary models allowing free intake of fat or sweet solutions to rodents [53] we are unaware of CAF diet protocols tailored for individual snack preference. Thus, the CAF-P protocol is a novel model of an obesogenic diet that could be used to explore how individual preference for foods influences the development of obesity. Finally, a more detailed exploration of the effect of different parameters of the CAF and CAF-P diets, including snack variety, portion size and frequency, on the individual susceptibility to obesity is necessary to determine the ability of Balb/c to maintain a lean phenotype under obesogenic conditions.

In conclusion, the results of this study describe a novel model of differential susceptibility to obesity in Balb/c mice fed two different variations of a cafeteria diet — standard and personalized. A key finding is that OR mice retain their low adiposity despite further increases in caloric intake by exposure to a CAF diet personalized to individual preferences. Complex phenotypes, such as susceptibility to obesity in rodents or SPA, can be dependent on breeding conditions [15]. Therefore, the specific characteristics of the OP/OR phenotype should be interpreted cautiously when compared other animal models. In summary our findings highlight the role of innate preferences and the interplay between changes in physical activity and hedonic intake underlying individual susceptibility to obesity.

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