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Inbred mouse strain survey of sucrose intake

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Abstract

Mouse strain differences for intake of sucrose and saccharin have been reported across studies, and some of these differences have been related to variants of the *Tas1r3* taste receptor gene. However, several methodological concerns remain, including use of relatively few strains and/or a limited number of palatable concentrations in previous analyses. The present study examined strain differences in sucrose intake among 11 inbred (A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL6/J, C57BL10/J, DBA/2J, SJL/J, SWR/J, 129P3/J) and one outbred (CD-1) mouse strains across nine different sucrose concentrations (0.0001–20%) using two-bottle 24-h preference tests which controlled for sucrose concentration presentation effects, sucrose and water bottle positions, and measurement of kilocalorie intake as sucrose or chow. A/J, C57BL/6J, CD-1 and SWR/J strains consumed the greatest (11.6–22 ml) amount of sucrose, whereas the A/J, C57BL/10J, SJL/J and SWR/J strains consumed the greatest (44–56%) percentages of kilocalories as sucrose. The AKR/J, CBA/J, C3H/HeJ and DBA/2J strains consumed the least (6.9–7.9 ml) amount of sucrose, and displayed lower (20–30%) percentages of kilocalories consumed as sucrose. Whereas A/J, C57BL/6J, C57BL/10J, CD-1, SWR/J and SJL/J strains all displayed the most pronounced compensatory decreases in chow intake as the percentage of kilocalories consumed as sucrose increased, the AKR/J, C3H/HeJ and DBA/2J strains failed to significantly alter chow intake even at high sucrose concentrations. There was a paucity of significant correlations in the percentage of sucrose intake between sucrose concentrations, but percentage of sucrose intake at lower concentrations did correlate with previous descriptions of saccharin intake and variants of the *Tas1r3* taste receptor gene. These data demonstrate clear mouse strain differences across a range of measures in sucrose intake across a wide range of concentrations, but caution against extrapolating between extremely high and low concentrations. The identification of strains with diverging abilities to regulate kilocalorie intake when presented with high sucrose concentrations may lead to the successful QTL mapping of this trait.

Keywords: Genetics; Hedonics; Taste; Post-ingestive factors; Preferences

1. Introduction

Systematic analyses of rodent strain differences are important sources regarding the genetic control of salt and nutrient intake (see review: [25]). These studies not only indicate widespread strain-dependent genetic variance, but may identify strains with divergent sensitivities for subsequent QTL analyses to localize chromosomal regions, and ultimately genes, critically involved in such differences. This approach has been very fruitful in the analysis of intake of salts (e.g., [1,5–7,30]), bitter tastants (e.g., [5,8,10,12,14,17,30]) and fats [4,28]. Simple sugars are potent stimulators of intake across a wide variety of species when included in solutions in a concentration-dependent manner (see review: [31]). Strain intake differences have been observed for sucrose (e.g., [3,8,16,18,24,29]) and saccharin (e.g., [8,9,11,16,18,20,21,26,30]). For example, a strong preference for a 0.1% saccharin solution relative to water was observed in BALB/cJ, C57BL/6J, I^S/Bi mice, but

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not in 101Bag/R1 mice [21]. Correspondingly, C57BL6/J mice displayed greater intake of five (0.005–1 M) glucose and sucrose concentrations than 101Bag/R1 mice [29], of a 0.1% saccharin solution than DBA/2J mice [11], and of a 4% sucrose solution than 129P3/J mice [3,30]. Genetic factors accounted for 78% and 83% of the genetic variation associated with consumption of 0.1% saccharin and 3% sucrose, respectively, in one outbred and seven inbred strains [24].

Most of these studies typically employed either a small number of strains or a limited number of palatable concentrations in their analyses. In compiling a list of strains, comparisons between studies are tenuous as strains and/or concentrations differed across studies, conceivably producing confounding methodological variables. Approaches using large numbers of strains have recently been adopted, demonstrating systematic differences in food, water and mineral intake as well as spout side preference in 28 strains [1,2]. Moreover, up to 26–30 strains of mice were examined for palatable intake in some studies. However, only single concentrations of saccharin (1.6 mM) or sucrose (50 mM) were employed [18,26]. More recently, 11 mouse strains were examined for alterations in sucrose intake, using seven supra-threshold (1–50%) concentrations [22]. However, important limitations in the genetic analysis of sweet intake remain due to the lack of important controls. These variables were addressed in the present study by controlling for the order of sucrose concentrations by exposing half of the mice to an ascending concentration order and the remainder to a descending concentration order, a variable of importance in prior work [13]. Bottle positions of the sucrose and water bottles were also systematically switched across animals and across strains, another variable of importance in prior work [2]. Moreover, careful measurement of chow intake was simultaneously assessed to determine strain differences in kilocalorie intake as a function of sucrose relative to chow. Additionally, the present study examined strain differences in sucrose intake among 11 inbred and one outbred (CD-1) strains across a far greater range of nine different sucrose concentrations (0.0001–20%) in two-bottle 24-h preference tests. These concentrations were selected based on their clear differentiations in sucrose intake in mice with distinct genetic genotypes [23]. By testing a sufficient number of randomly chosen inbred strains, the present study design also allows for the valid estimation of genetic correlations [15]. The demonstration of a genetic correlation implies the involvement of common (although not necessarily identical) physiological substrates. Thus, genetic commonality in the percentage of sucrose intake among sucrose concentrations was tested. Previously, Lush [18] reported on intake for 1.6 mM saccharin solution in 30 inbred mouse strains, including some of those tested in the present study.

Subsequently, Reed and co-workers [26] identified the sequence variants of the previously identified *Tas1r3* taste receptor gene which codes for the protein associated with this preference. Their relevance to saccharin intake was demonstrated by genotyping these polymorphisms in 30 inbred strains and comparing their allelic frequencies for these variants with their saccharin preference. We therefore also assessed the relationship between percentages of sucrose intake in the present study with previously reported saccharin intake for these strains, and between strain variation in the percentage of sucrose intake observed in the present study with polymorphisms of the *Tas1r3* taste receptor gene associated with percentage of 1.6 mM saccharin intake. For all correlations, coefficients of covariation were obtained across a range of sucrose concentrations.

2. Methods

2.1. Subjects

Outbred (CD-1, Charles River Laboratories, Wilmington, MA; $n=20$) and inbred A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, C57BL/10J, DBA/2J, SJL/J, SWR/J, 129P3/J (Jackson Laboratories, Bar Harbor, ME; $n=9–10$ each) male mice (12 weeks of age) were initially acclimated to the Queens College vivarium for 1 week in group (5 per cage) housing. Then, each animal was housed individually in plastic cages ($30\times20\times15$ cm) throughout the entire study, and maintained on a 12 h light/12 h dark cycle (lights off at 2000 h) at a constant temperature of 22 °C.

2.2. Sucrose intake procedure

All procedures were approved by the Queens College Institutional Animal Care and Use Committee. Initially, each animal was provided with a pre-weighed ration (~20 g) of Purina Mouse chow (5.3 kcal/g) and two calibrated and preweighed (100 ml capacity, +0.1 g=+0.1 ml; Lab Products, Seaford, DE) sipper tubes each filled with water (~40 ml each). Each animal of each strain was assessed for chow and water intakes from each individual bottle every 24 h over 4 days. Whereas chow spillage was measured and adjustments of intake were made accordingly, it should be noted that there was no systematic measurement of water or sucrose spillage, although very infrequent malfunction of a sipper tube (resulting in large spillage) was noted, and the data discarded. Body weights of the animals were periodically measured throughout the paradigm, and a mean body weight was chosen for each animal of each strain for further analysis. The position of the two water bottles were switched across animals and across strains every 24 h according to a left (L)–right (R)–R–L and R–L–L–R position respectively to minimize potential bottle position preference effects [2]. Following baseline, each mouse of each strain received chow, one bottle of water and one bottle of sucrose each day. Nine sucrose concentrations were tested: 0.0001%, 0.001%, 0.01%, 0.1%, 1.0%, 2.5%, 5.0%,

10% and 20% in these two-bottle preference tests. Half of the mice of each strain were tested in an ascending sucrose concentration order, and the remaining half were tested in a descending order with sucrose bottle position systematically controlled [13]. Chow, sucrose and water intakes (+0.1 g) were measured daily for each concentration of sucrose.

2.3. Statistics

One-way analyses of variance were performed to assess whether any pre-existing differences in body weight and in total baseline water intake and chow intakes were observed across strains. In this and all subsequent analyses involving chow, data from 11 of the 12 tested strains are presented, as the 129P3/J strain produced a great deal of spillage that produced accurate measurement problems. To assess sampling of the two water bottles under baseline conditions, a two-way randomized block analysis of variance was also performed with strains as the between-subject variable and water intake from the two bottles as a repeated measure. In assessing alterations in sucrose relative to water intake, a three-way randomized block analysis of variance was performed with the 12 strains as the between-subject variable, the 10 (baseline and 9 sucrose concentration) conditions as a within-subject variable, and the intake from the sucrose and water bottles as a second within-subject variable. Further, to assess order effects upon sucrose intake, another set of three-way randomized-block analyses of variance systematically compared within each strain those mice that received an ascending order of sucrose concentrations with those that received a descending order. Two-way randomized-block analyses of variance were also systematically performed across strains and across sucrose concentrations to assess changes in the percentage of sucrose consumed, the total amount of chow intake, and the percentage of kilocalories consumed as sucrose. Finally, since there were significant differences in body weight across strains (Table 1), a two-way randomized block analysis of variance was performed with the 12 strains as the between-subject variable and the 10 conditions as a within-subject variable for transformed sucrose intake per 30 g of body weight. Tukey comparisons ($p<0.05$) were performed in the presence of significant effects relative to corresponding baseline values within strains.

All correlations were calculated using Pearson product-moment correlation coefficients (r) subject to Bonferroni correction for multiple comparisons. For all analyses, the following sucrose concentrations were considered: 0.01%, 0.1%, 1.0%, 2.5%, 5.0%, and 10.0%. Lower and higher concentrations were not subject to correlation analyses since the respective uniformly low and high percentages of sucrose intake at these concentrations could restrict the range of values and underestimate correlations. One exception was our assessment of the correlation between 1% and 2.5% sucrose intake and the total (sucrose and chow) kilocaloric intake of mice offered a 20% sucrose solution, and between 1% and 2.5% sucrose intake and 20% sucrose intake (per 30 g/body weight). For the genetic codetermination between sucrose concentrations, the proportion of sucrose intake relative to total fluid intake (percentage of sucrose intake) for each strain at each sucrose concentration correlated with each other. To assess the relationship between sucrose and saccharin intake, percentage of sucrose intake for each sucrose concentration was correlated with percentage of 1.6 mM saccharin intake as previously reported [18]. The covariation between sucrose intake and *Tas1r3* polymorphisms previously described [26] was achieved by assigning a gene dose value of 1 or 2 to each strain displaying an allele associated with high and low saccharin preference, respectively. *Tas1r3* variants assessed were those with the greatest degree of statistical association with saccharin intake (nucleotide positions: −791 (5′region), +135 (Exon 1), and +179 (Exon 1)). Since all alleles associated with high and low saccharin intake were represented by a gene dose score of 1 or 2, respectively, correlation coefficients were determined for each sucrose concentration once to minimize type I error and were generalized to all three polymorphisms. Data from all inbred strains were included in the correlation analyses except C57BL/10J as they have not been genotyped for *Tas1r3* variants and their high genetic similarity to C57BL/6J may overestimate genetic correlations.

Table 1
Baseline water (ml, ±S.E.M.) and chow (g, ±S.E.M.) intake and body weight (g, ±S.E.M.) in 12 mouse strains

Strain	Water (ml)	Chow (g)	Body weight (g)
A/J	5.6 (0.7)	4.4 (0.3)	26.2 (1.4)
AKR/J	5.7 (0.6)	4.6 (0.1)	33.6 (1.4)
BALB/cJ	5.6 (0.1)	5.4 (0.2)[a]	27.9 (0.8)
C57BL/6J	4.9 (0.3)	4.1 (0.1)	27.9 (0.6)
C57BL/10J	5.5 (0.3)	3.7 (0.1)	26.8 (0.5)
CBA/J	5.3 (0.3)	3.8 (0.1)	32.3 (1.5)
CD-1	7.8 (0.4)[a]	5.8 (0.3)[a]	37.7 (0.8)[a]
C3H/HeJ	5.2 (0.4)	4.2 (0.1)	28.4 (0.5)
DBA/2J	5.1 (0.1)	4.4 (0.2)	27.0 (0.8)
SJL/J	5.7 (0.3)	3.5 (0.2)	25.6 (0.2)
SWR/J	7.3 (0.3)[a]	4.6 (0.2)	27.2 (0.3)
129P3/J	7.1 (0.4)[a]	n.a.	30.5 (0.4)

[a] Significantly greater relative to all other unmarked strains in column.
n.a.: not available.

3. Results

3.1. Baseline values in water and chow intake

Significant differences were observed among mouse strains in total baseline water ($F(11,117)=7.40$, $p<0.0001$) and chow ($10,108)=13.46$, $p<0.0001$) intakes. As summarized in Table 1, baseline water intake was greatest in CD-1, SWR/J, and 129P3/J strains, with significantly less water intake observed in all other strains. Baseline chow intake was again greatest in CD-1 mice with similar intake

observed for the BALB/cJ strain (Table 1). Significant differences were observed among mouse strains in body weight ($F(11,117)=30.21$, $p<0.0001$). Body weight was significantly greater in CD-1 mice relative to the other 11 strains which in turn failed to display significant differences among one another (Table 1). Analysis of two-bottle baseline water intake revealed significant differences across strains ($F(11,209)=26.04$, $p<0.0001$) and for the interaction between strains and fluid choice ($F(11,209)=3.29$, $p<0.0001$), but not for intake for the two fluids ($F(1,19)=0.10$, n.s.). Importantly, all 12 strains displayed similar patterns of sampling of the two water bottles during baseline (BL) testing (Fig. 1), indicating that preferences described for intake of different concentrations of sucrose were not due to some underlying intra-strain preference for intake from one water bottle.

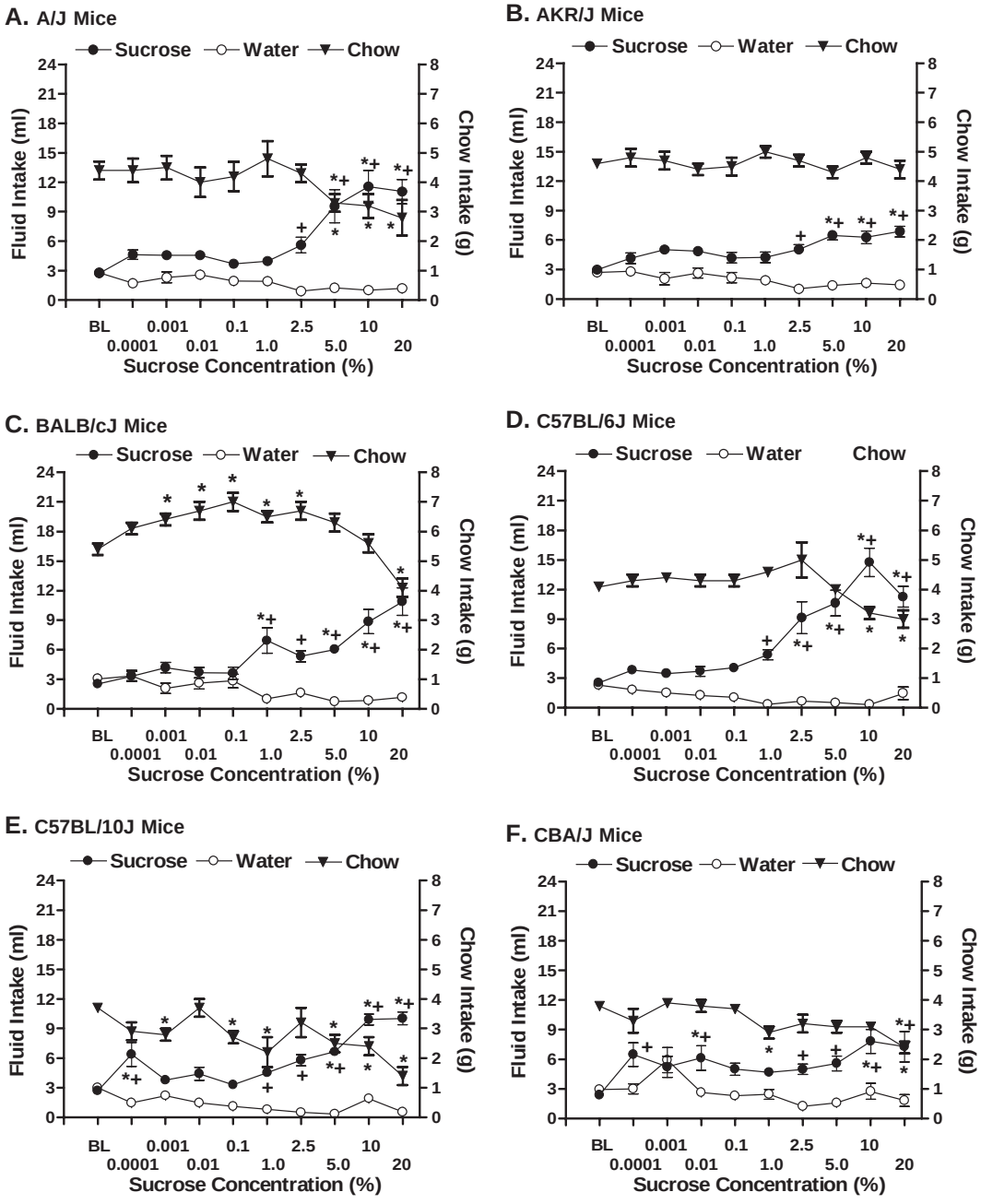

Fig. 1. Alterations in sucrose (left ordinate, mean, ±S.E.M.), water (left ordinate, mean, ±S.E.M.) and chow (right ordinate, mean, ±S.E.M.) intake across baseline and nine different sucrose concentrations in one outbred (CD-1) and 11 inbred (A/J, AKR/J, BALB/cJ, C57BL/6J, C57BL/10J, CBA/J, C3H/HeJ, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice. In this and all subsequent figures, the asterisks (*) denote a significant difference in intake relative to corresponding baseline conditions, and the crosses (+) denote a significant difference in sucrose intake relative to corresponding water intake at that concentration (Tukey comparisons, $p<0.05$). 129P3/J mice had large spillage precluding careful measurement of chow intake.

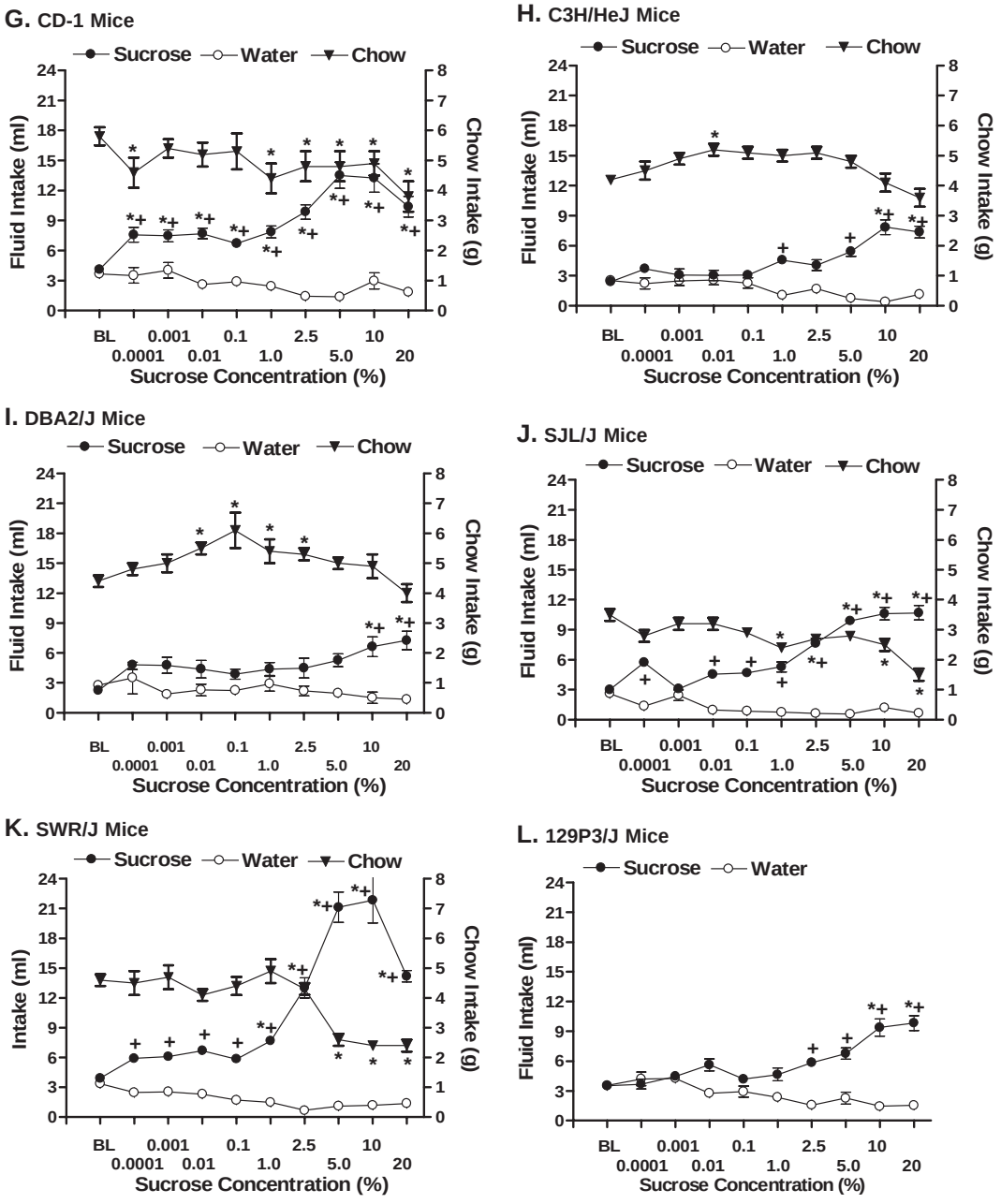

Fig. 1 (*continued*).

3.2. Sucrose and water intake

In analyzing sucrose and water intake across strains and sucrose concentrations, significant differences in intake were observed among strains ($F(11,209)=43.46$, $p<0.0001$), across concentrations ($F(9,171)=239.45$, $p<0.0001$), between the two fluids ($F(1,19)=1151.48$, $p<0.0001$), and for the interactions between strains and concentrations ($F(99,1881)=27.45$, $p<0.0001$), strains and fluids ($F(11,209)=53.34$, $p<0.0001$), concentrations and fluids ($F(9,171)=378.75$, $p<0.0001$) and among strains, concentrations and fluids ($F(99,1881)=17.71$, $p<0.0001$). Significant differences in chow intake were observed among strains ($F(10,190)=52.63$, $p<0.0001$), across concentrations ($F(9,171)=137.44$, $p<0.0001$) and for the interaction between strains and concentrations ($F(90, 1710)=12.43$, $p<0.0001$). The effects of sucrose concentration on intake were not generally attributable to the order of sucrose concentration presentation, as mice exposed to ascending and descending orders generally failed to differ in intake. However, CBA/J mice exposed to the ascending order of sucrose concentrations consumed significantly more sucrose at the 0.0001–0.01% concentrations than the same strain exposed to the descending order of sucrose concentrations, indicating the importance of using this order control [13].

Among strains, only CD-1 and SWR/J mice significantly increased their sucrose intake relative to either corresponding water intake at every sucrose concentration or to baseline water intake from the "sucrose" bottle (Fig. 1G and K). For all other strains except the DBA/2J strain, significantly greater sucrose intake relative to water intake was also observed at higher sucrose concentrations (1% or 2.5–20%), but significant increases in sucrose intake at lower concentrations was strain-dependent (Fig. 1A–F, H, J, L). DBA/2J mice significantly consumed more sucrose than water at only the highest (10% and 20%) sucrose concentrations (Fig. 1I). Thus, inter-strain variability for total sucrose intake was observed across the entire range of sucrose concentrations.

An analysis of variance examining sucrose intake per 30 g of body weight revealed significant differences among strains ($F(11,209)=61.07$, $p<0.0001$), among sucrose concentrations ($F(9,171)=430.09$, $p<0.0001$) and for the interaction between strains and concentrations ($F(99,1881)=32.98$, $p<0.0001$). Table 2 summarizes the strain differences in body weight-corrected sucrose intake with the SWR/J strain displaying significantly greater intake than all other strains at most of the sucrose concentrations. In turn, the C57BL/6J strain displayed significantly greater intake at the four higher concentrations than the DBA/2J, C3H/HeJ, 129P3/J, AKR/J and CBA/J strains, at the three higher concentrations than the BALB/cJ and C57BL/10J strains, and at some concentrations than the CD-1 (10–20%), A/J(2.5%) and SJL/J (10%) strains. Moreover, the SJL/J strain displayed significantly greater intake at the four higher concentrations than the DBA/2J, C3H/HeJ, 129P3/J, AKR/J and CBA/J strains, at the three higher concentrations than the BALB/cJ strain and at some concentrations than the CD-1 (10–20%), C57BL/6J (2.5%) and C57BL/10J (5%) strains. Further, the A/J strain displayed significantly greater intake at the three higher concentrations than the DBA/2J, C3H/HeJ, BALB/cJ, 129P3/J, AKR/J and CBA/J strains and at some concentrations than the CD-1 (10–20%), C57BL/6J (2.5%) and C57BL/10J (5%) strains. The major differences in the pattern of estimated sucrose intake using raw or weight-adjusted intake occurred for the heaviest CD-1 mice (Table 2 vs. Fig. 1G).

Table 3
Strain summary of maximal sucrose intake (ml), maximal percentage of fluid intake ingested as sucrose, and maximal percentage of kilocalorie intake ingested as sucrose (±S.E.M.) with the sucrose concentration for each in brackets

Strain	Sucrose intake (ml)	% Fluid intake as sucrose	% Kilocalorie intake as sucrose
A/J	11.6 (1.7) [10%]	91 (1.9) [10%]	44 (0.09) [20%]
AKR/J	6.9 (0.5) [20%]	83 (3.7) [5%]	20 (0.02) [20%]
BALB/cJ	10.9 (1.4) [20%]	90 (2.1) [10%]	28 (0.03) [20%]
C57BL/6J	14.8 (1.5) [10%]	98 (0.4) [10%]	37 (0.03) [20%]
C57BL/10J	10.0 (0.7) [20%]	95 (0.8) [20%]	56 (0.05) [20%]
CBA/J	7.8 (1.2) [10%]	79 (6.6) [2.5%]	30 (0.06) [20%]
CD-1	13.5 (1.3) [5%]	90 (1.6) [5%]	32 (0.03) [20%]
C3H/HeJ	7.9 (0.7) [10%]	95 (1.4) [10%]	25 (0.02) [20%]
DBA/2J	7.3 (0.9) [20%]	88 (3.8) [10%]	22 (0.02) [20%]
SJL/J	10.7 (0.7) [20%]	95 (0.8) [5%]	54 (0.05) [20%]
SWR/J	21.9 (2.3) [10%]	95 (0.6) [5%]	48 (0.03) [20%]
129P3/J	9.8 (0.8) [20%]	87 (2.3) [10%]	n/a

129P3/J mice had large spillage precluding careful measurement of chow intake.

The raw data predicted very well the transformed data considering percentage of sucrose intake as a function of total fluid intake across sucrose concentrations and strains (Table 3). Significant differences in the percentage of fluid intake consumed as sucrose were observed among strains ($F(11,209)=23.89$, $p<0.0001$), across concentrations ($F(9,171)=235.88$, $p<0.0001$), and for the interaction between strains and concentrations ($F(99,1881)=6.43$, $p<0.0001$). Consistent with the data analyzing the amount of sucrose consumed, the percentage of intake consumed as sucrose was significantly higher in CD-1 and SWR/J mice at every concentration, and followed a fairly monotonic function (Fig. 2G and K). Except for C3H/HeJ mice, all other strains displayed variability in the significant sucrose percentage effects at all but the lowest (0.0001–0.1%)

Table 2
Alterations in sucrose (mean, ±S.E.M.) intake, each corrected for 30 g of body weight, across baseline and nine different sucrose concentrations in one outbred (CD-1) and 11 inbred (A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57BL/10J, CBA/J, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice

Strain	0.0001%	0.001%	0.01%	0.1%	1.0%	2.5%	5.0%	10.0%	20.0%
A/J	0.59 (0.07)	0.59 (0.03)	0.58 (0.04)	0.47 (0.06)	0.51 (0.05)	0.72*	1.23* (0.22)	1.50* (0.22)	1.42* (0.16)
AKR/J	0.43 (0.06)	0.50 (0.03)	0.49 (0.05)	0.44 (0.07)	0.44 (0.06)	0.52 (0.06)	0.65* (0.05)	0.65* (0.09)	0.71* (0.08)
BALB/cJ	0.41 (0.06)	0.51 (0.07)	0.45 (0.07)	0.44 (0.08)	0.81* (0.13)	0.64* (0.07)	0.73* (0.05)	1.08* (0.17)	1.30* (0.15)
C3H/HeJ	0.43 (0.05)	0.36 (0.07)	0.36 (0.06)	0.36 (0.05)	0.54 (0.04)	0.48 (0.06)	0.64* (0.06)	0.92* (0.08)	0.90* (0.06)
C57BL/6J	0.46 (0.05)	0.41 (0.05)	0.44 (0.06)	0.49 (0.05)	0.65* (0.06)	1.10* (0.19)	1.27* (0.16)	1.78*(0.18)	1.36* (0.14)
C57BL/10J	0.84* (0.19)	0.47 (0.05)	0.55 (0.08)	0.41 (0.03)	0.55 (0.04)	0.72* (0.06)	0.83* (0.05)	1.23* (0.06)	1.25* (0.07)
CBA/J	0.78* (0.17)	0.62* (0.12)	0.64* (0.13)	0.54* (0.08)	0.50 (0.06)	0.53 (0.06)	0.58* (0.07)	0.86* (0.16)	0.78* (0.17)
CD-1	0.67* (0.07)	0.66* (0.05)	0.68* (0.04)	0.60* (0.04)	0.69* (0.05)	0.87* (0.06)	1.12* (0.12)	1.17* (0.12)	0.91* (0.09)
DBA/2J	0.59* (0.05)	0.58* (0.09)	0.54 (0.10)	0.48 (0.06)	0.54 (0.07)	0.55 (0.11)	0.65* (0.05)	0.82* (0.11)	0.91* (0.12)
SJL/J	0.75* (0.06)	0.40 (0.04)	0.59 (0.03)	0.61 (0.04)	0.68 (0.06)	0.99* (0.06)	1.29* (0.04)	1.38* (0.08)	1.39* (0.09)
SWR/J	0.72 (0.05)	0.75 (0.05)	0.82* (0.05)	0.71 (0.05)	0.94* (0.05)	1.59* (0.12)	2.59* (0.19)	2.68* (0.28)	1.74* (0.07)
129P3/J	0.40 (0.05)	0.49 (0.04)	0.61 (0.06)	0.46 (0.05)	0.51 (0.07)	0.64 (0.05)	0.74* (0.05)	1.03* (0.09)	1.07* (0.08)

* Significant difference from corresponding baseline value ($p<0.05$).

sucrose concentrations (Fig. 2A–F, I, J, L). For the C3H/HeJ strain, significant sucrose percentage effects were detected only at the five highest sucrose concentrations (Fig. 2H).

3.3. Kilocalorie intake as sucrose and chow

Significant differences in the percentage of kilocalorie intake consumed as sucrose were observed among strains ($F(10,190)=22.88$, $p<0.0001$), across concentrations ($F(8,152)=957.13$, $p<0.0001$), and for the interaction between strains and concentrations ($F(80, 1520)=18.54$, $p<0.0001$). Although all mouse strains consumed a considerable amount of their kilocalories as sucrose, particularly at the higher concentrations, they showed systematic differences in the percentage of kilocalories consumed as sucrose (Table 3, Fig. 2). Thus, approximately 50% of total kilocalorie consumption as sucrose was observed in C57BL/10J, SJL/J and SWR/J strains (Fig. 2E, J, K), and over 40% consumption was observed in the A/J strain (Fig. 2A). Moderate (~30%) consumption was noted in BALB/cJ, C57BL/6J, CBA/J, CD-1 and C3H/HeJ strains (Fig. 2C, D, F–H), and lower (~20%) consumption was observed in AKR/J and DBA/2J strains (Fig. 2B, I).

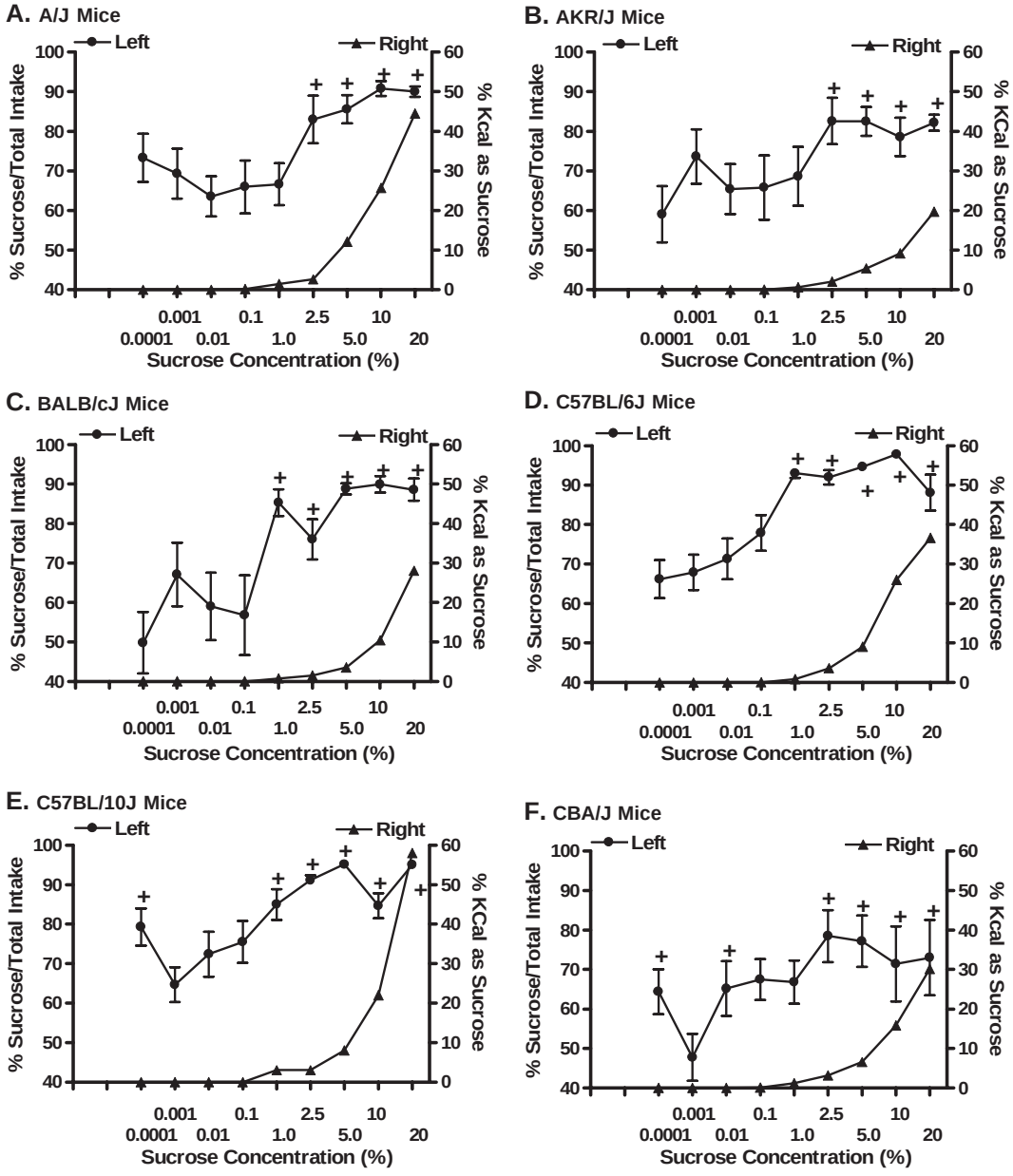

Fig. 2. Alterations in the percentage of sucrose over total intake (left ordinate, mean, ±S.E.M.) and the percentage of kilocalories consumed as sucrose (right ordinate, mean, ±S.E.M.) across the nine different sucrose concentrations in one outbred (CD-1) and 11 inbred (A/J, AKR/J, BALB/c/J, C57BL/6J, C57BL/6J, CBA/J, C3H/HeJ, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice. The large spillage by 129P3/J mice precluded measurement of chow intake and therefore, measurement of the percentage of kilocalories consumed as sucrose.

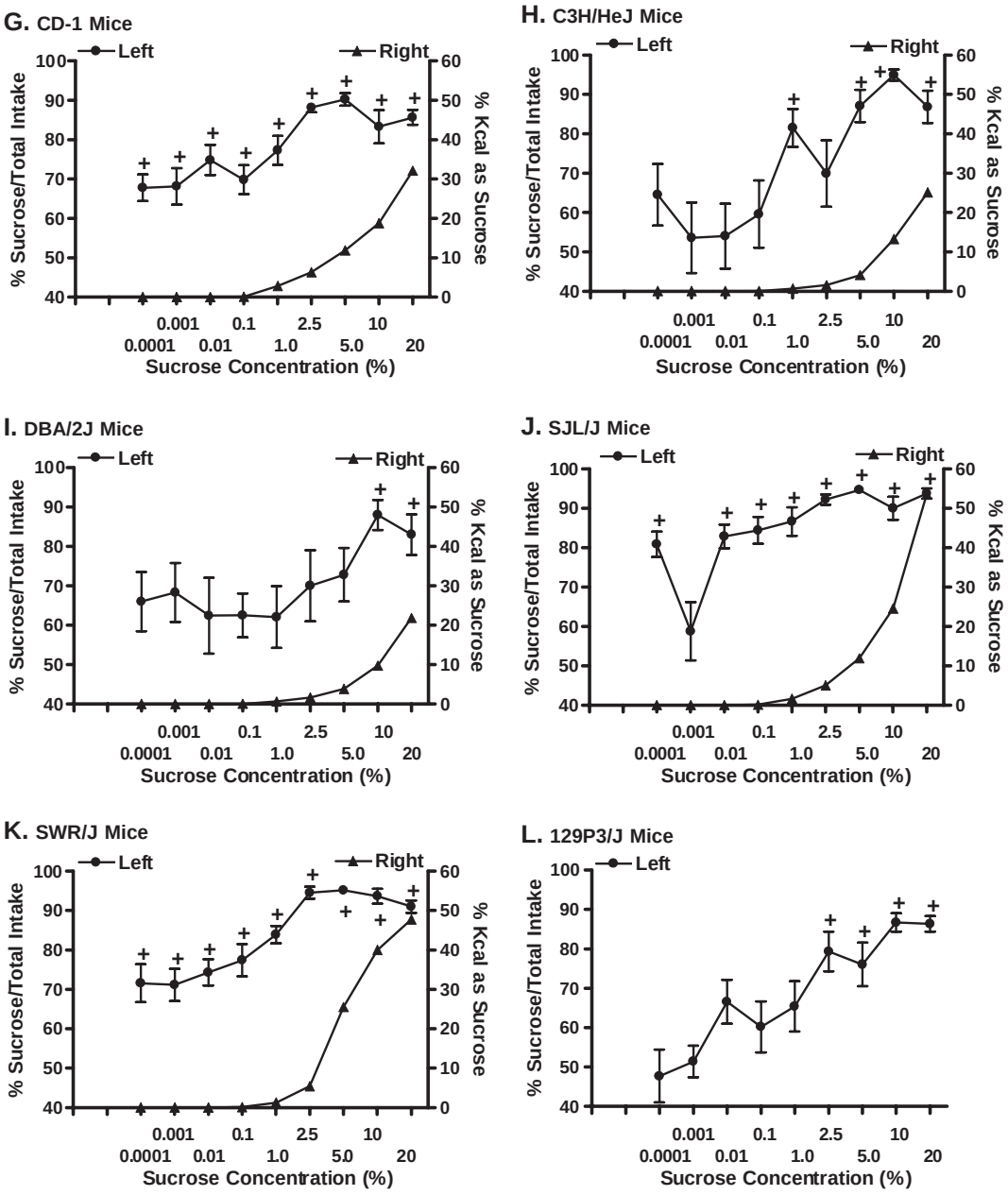

Fig. 2 (*continued*).

Increased sucrose consumption at the high sucrose concentrations also resulted in a compensatory decrease in chow intake in some strains (A/J, C57BL/6J, C57BL/10J, CBA/J, CD-1, SJL/J, and SWR/J). Except for CD-1 mice in which significant reductions in chow intake was already reduced at a sucrose concentration as low as 1% (Fig. 1G), significant reductions in chow intake typically occurred at higher sucrose concentrations (5–20%: Fig. 1A, D–F, J, K). In contrast, chow intake failed to vary at any sucrose concentrations in the AKR/J strain (Fig. 1B), and actually significantly increased across ranges of lower sucrose concentrations in BALB/cJ, C3H/HeJ, and DBA/2J mice (Fig. 1C, H, I).

3.4. Correlational data

Table 4 displays the pairwise correlation coefficients between some of the sucrose concentrations. Significant covariation was only transiently observed, typically between some of the lower concentrations. Sucrose concentrations of 1% and 10% were not correlated with any other sucrose concentration. There was a similar lack of significant correlation between percentage of sucrose intake and percentage of saccharin intake as previously reported by Lush [18]. As evident from Table 5, the percentage intake of a previously reported [18] 1.6 mM saccharin solution was correlated only with intake of a 0.1% sucrose solution

Table 4
Pearson product–moment correlation coefficients between sucrose concentrations for percentage sucrose intake in 10 inbred mouse strains

Sucrose concentration	0.01%	0.1%	1.0%	2.5%	5.0%
0.01%	–				
0.1%	0.91*	–			
1.0%	0.36	0.51	–		
2.5%	0.86*	0.86*	0.53	–	
5.0%	0.49	0.62	0.91*	0.71	–
10.0%	0.09	0.21	0.63	0.24	0.62

* Significant correlation after Bonferroni corrections ($p<0.05$).

measured in the present study. Correlation coefficients (Table 5) between strain variation in the percentage of sucrose intake and three *Tas1r3* variants [26] appeared to be sucrose concentration-dependent, with instances of significant covariation observed at lower sucrose concentrations. The intake of a 20% sucrose concentration or total kilocaloric intake during its availability was not significantly correlated with either 1% or 2.5% sucrose intake (data not shown).

4. Discussion

Strong and systematic strain differences were observed for sucrose intake especially as functions of the total amount of sucrose consumed and the percentage of total kilocalories consumed as sucrose. In this regard, the A/J, C57BL/6J, CD-1 and SWR/J strains consumed the greatest (11.6–22 ml) total amounts of sucrose. When adjusted for body weight, the A/J, C57BL/6J J, SJL/J and SWR/J strains persisted in consuming the greatest amounts of sucrose per 10 g of body weight (4.2–8 ml/10 g BW). Further, the A/J, C57BL/10J, SJL/J and SWR/J strains consumed the greatest (44–56%) percentages of kilocalories consumed as sucrose. The BALB/cJ and 129P3/J strains displayed intermediate responsiveness. Alternatively, the AKR/J, CBA/J, C3H/HeJ and DBA/2J strains appeared to consume the least (6.9–7.9 ml) amount of sucrose, and displayed lower (20–30%) percentages of kilocalories consumed as sucrose. Interestingly, the A/J, C57BL/6J, C57BL/10J, CD-1, SJL/J and SWR/J strains all displayed the most pronounced compensatory decreases in chow intake as the percentage of kilocalories consumed as sucrose increased. Whereas BALB/cJ and CBA/J mice displayed this effect at the highest sucrose concentration, the AKR/J, C3H/HeJ and DBA/2J strains failed to significantly alter chow intake at any of the sucrose concentrations.

The present study differed from previous studies in terms of the number of strains [12] tested across a wide [9] range of both "sub-threshold" (0.0001–0.1%) and "supra-threshold" (1–20%) sucrose concentrations using two-bottle 24-h preference tests. Moreover, the order of presentation of sucrose concentrations was controlled as suggested from previous work [13], and was found to be an important variable for only one (CBA/J) strain. Although sucrose and water bottle positions were also controlled given the previous [2] relevance of this variable, the bottle positions were switched every 24 h. It is important to note a potential limitation of our testing procedure in that it differs from a technique of presenting solutions for two consecutive days ("the 48 h test") and switching the sides of the presentation between the first and second day. Although the 1-day test procedure may have added some noise to the data, we do not believe that this invalidates the results. Finally, systematic measurement of chow, water and sucrose intake allowed the determination of strain differences in kilocalorie intake consumed as sucrose as well as systematic changes in chow intake across sucrose concentrations. Among the A/J, C57BL/6J, CD-1 and SWR/J strains showing the greatest magnitude of sucrose intake, the CD-1 and SWR/J strains also displayed the greatest sensitivities to sucrose, showing significantly greater consumption across all nine sucrose concentrations relative to the corresponding water ration. In contrast, significantly greater sucrose consumption occurred for A/J and C57BL/6J strains at the 2.5% and 5% sucrose concentration respectively. Among the AKR/J, CBA/J, C3H/HeJ and DBA/2J strains showing the smallest magnitude of sucrose intake, the C3H/HeJ and DBA/2J strains also displayed the least sensitivities to sucrose, showing significantly greater consumption across the two highest (10–20%) sucrose concentrations only. A number of previous studies have employed the percentage of sweetener consumed as a function of total fluid intake as a measure of preference (e.g., [9,11,18,22]). In the present study however, strains that showed both larger (e.g., C57BL/6J, C57BL/10J, SWR/J, SJL/J) and smaller (e.g., C3H/HeJ) magnitudes of sucrose intake invariably showed very high (>95%) preferences for sucrose, underlining the importance of studying more strains across greater sucrose concentrations.

Interestingly, significant pairwise correlation coefficients between sucrose concentrations were only transiently observed. It is unlikely that the estimation of covariance was underestimated by restricting the range at the highest (10%) and lowest (0.01%) sucrose concentrations since significant strain differences were evident and the range of

Table 5
Pearson product–moment correlation coefficients between sucrose concentration and percentage saccharin intake and three polymorphisms in the *Tas1r3* taste receptor gene in 10 inbred mouse strains

	% Saccharin intake[a]	*Tas1r3* polymorphisms[b]
Sucrose concentration		
0.01%	0.71	0.83*
0.1%	0.81*	0.91*
1.0%	0.76	0.74
2.5%	0.73	0.86*
5.0%	0.74	0.77
10.0%	0.51	0.50

[a] See Lush [18] for % saccharin intake data.
[b] See Reed et al. [26] for Tas1r3 polymnorphims.
* Significant correlation after Bonferroni corrections ($p<0.05$).

differences in the percentage of sucrose intake identical to those obtained for lower and middle concentrations. Furthermore, more moderate 1.0% and 5.0% sucrose concentrations also showed little if any significant correlations. Since we used a sufficient number of randomly chosen inbred strains, allowing for the estimation of genetic correlations [15], the data suggest that the genetic, and ultimately physiological, regulation of sucrose intake may differ across a range of concentrations.

As noted above, large [26–30] numbers of mouse strains were recently examined for sweet intake using only single saccharin (1.6 mM) or sucrose (50 mM) concentration [18,26], limiting generalizability of effects. Lush [18] found that the pattern of strong preferences for saccharin and sucrose at these single concentrations were greater in A/J, C57BL/6J, C57BL/10J and SWR/J strains (73–97%) than in AKR/J, CBA/J, C3H/HeJ, DBA/2J and 129P3/J strains (51–61%). We correlated our data at various sucrose concentrations with that of Lush [18] at the 1.6 mM saccharin concentration (Table 5) and found that the correlation between sweetener concentrations were not uniformly significant. With regards to saccharin intake, it is notable that a significant coefficient was obtained with only the relatively low 0.1% sucrose concentration. That is, the relationship between two sweeteners at only very low concentrations of each is consistent with our conclusion above that higher sucrose concentrations may be under differential genetic control than those mediating relatively lower concentrations. In support of genetic commonality at lower sweetener concentrations, Capeless and Whitney [9] found order of effects similar to those observed in the present study for strain preference scores in 129P3/J, C57BL/6J, BALB/cJ, C3H/HeJ, and DBA/2J mice across four saccharin concentrations [8,11]. Furthermore, previous examination [22] of only supra-threshold sucrose concentrations revealed little consistent strain-specific differences in sucrose intake except for 129P3/J mice showing the smallest magnitudes of responses. Therefore, previous studies show strong and similar patterns of results to the present findings.

We also observed differential strain sensitivity to compensatory decreases in chow intake as the percentage of kilocalories consumed as sucrose increased. Whereas the A/J, C57BL/6J, C57BL/10J, CD-1, SJL/J and SWR/J strains all displayed the most pronounced compensatory decreases in chow intake, the AKR/J, C3H/HeJ and DBA/2J strains failed to significantly alter chow intake at any of the sucrose concentrations. This very rapid compensation to chow in the presence of sucrose suggests that such strains be may be displaying either a sensitivity to energy intake or greater ability to both adapt and respond. Divergent responders may be a model for studying and identifying genetic substrates associated with this ability to regulate kilocalorie intake across a variety of energy sources. Such data might predict the ability of chronic exposure to concentrated sucrose as an energy source to chow to increase weight gain, obesity and diabetic symptoms in non-compensating strains relative to compensating strains.

Extreme responding strains currently identified for several sucrose intake-related variables may serve as progenitors for quantitative trait loci (QTL) analysis and subsequently, the identification of trait relevant genes. Previous QTLs for saccharin and sucrose intake, including Prp [8], Sac [11,18,19], and Tas1r3 [16] have been localized to distal chromosome 4 using C57BL/6J crosses with DBA/2J or 129P3/J mice. Interestingly, these strains did not display the most divergent responses in sucrose intake from the 11 strains evaluated in this study. Furthermore, our correlations between the percentage of sucrose intake and *Tas1r3* taste receptor gene variants were not uniformly observed across sucrose concentrations (Table 5). Conversely, and true as well for the correlation between the percentage of sucrose intakes and *Tas1r3* taste receptor gene variants, it is possible we have underestimated the correlation by reducing statistical power when correcting for multiple comparisons. In particular, many coefficient values exceeded 0.70. We do not believe this is true for the correlation between sucrose concentrations since many of those coefficients are quite low and would most likely fail to achieve significance even without Bonferroni corrections. Nonetheless, correlation coefficients between the percentage of sucrose intake of a 10% sucrose solution and the percentage intake of a 1.6 mM saccharin or sucrose solution of any concentration, or with *Tas1r3* taste receptor gene variants, were uniformly low. These particular correlations support our assertion above that distinct genetic mechanisms may underlie the intake of solutions with high and low sweetener concentration, and argue that a comprehensive understanding of intake of sweeteners like sucrose will require study across a range of concentrations.

Sucrose intake is a complex behavior with separate and dissociable orosensory and post-ingestive mechanisms (see review: [27]), and thus in all likelihood, is under polygenic control. It is likely that QTL analyses with more divergent sensitivities such as those characterized in the present study will allow for identification of additional trait-relevant QTLs, including those with smaller contributions to the overall genotypic variance. Additionally, using strains presently identified as good or poor regulators of chow, and thus overall, caloric intake during high sucrose ingestion may provide genetic insight into to obesity-related problems.

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