



## **A Comprehensive Description of Intake of Diverse Foods by Rats (*Rattus norvegicus*) Selectively Bred on a Taste Phenotype**

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Eating is a central feature of the lives of opportunistic omnivores such as humans and Norway rats. Yet in most laboratory research with *Rattus norvegicus*, the food landscape is monotonous, and the studies utilizing a variety of foods shed little light on their intake of individual foods or choice behavior. The present study provides the most comprehensive description to date of female and male laboratory rats' intake of foods that they and humans encounter outside of the laboratory. In 11 experiments, test foods included varieties of peanut butter, cheese, cookies, meat, chocolate, fruits, and vegetables. Rats were given commercial products or custom versions that controlled for the proportion of calories from fat and caloric density. These foods were presented to the rats either one or two foods at a time. A final experiment examined pure macronutrient self-selection. Intraspecies diversity was modeled with rat lines selectively bred on a taste phenotype. All groups voluntarily ate every food, with their intake (in grams) highest for vegetables and lowest for pure macronutrients. When Low- (LoS) and High-Saccharin-Consuming (HiS) rats differed, LoS rats ate more meat and fat and were choosier whereas HiS rats ate high-carbohydrate foods more avidly; exceptions and sex-dependent differences occurred. Using these results to enrich the food landscape for laboratory rats can enhance the comparative study of food intake and its relation to other behavioral systems.

*Keywords:* caloric density, dietary propensities, fat content, omnivory, rats, saccharin phenotype

What animals eat can shape and reflect their emotions and behaviors (Rozin & Todd, 2016; Singh, 2014). For example, a Western diet high in fat, sugar, and calories is associated with biomarkers for mood disorders and impaired eating regulation in humans (Davidson et al., 2019; Jacka et al., 2010). This diet also influences anxiety, behavioral depression, and performance in memory tasks in rodents and monkeys (Acosta et al., 2017; Attuquayefio et al., 2016; Chilton et al., 2011; Cordner & Tamashiro, 2015; Ferreira et al., 2018; Wait et al., 2021). In addition, taste and dietary preferences can be markers for activity, mood, temperament, and personality in humans and other species (e.g., Ha et al., 2019; Kaukonen et al., 2019; Sagioglou & Greitemeyer, 2016; Spinelli et al., 2018). Understanding why animals eat more of certain types of foods than other types is important for scientific and practical reasons.

Achieving that understanding is challenged by the complexity of influences on food intake that are amenable to study at levels of organization from subcellular to ecological and on time scales from evolutionary to situational (e.g., Baumgartner et al., 2020; Benoit et al., 2010; Breslin, 2013; Raynor & Epstein, 2001; Spector & Glendinning, 2009). A conventional way of tackling this challenge in research with laboratory rats has been the experimental isolation of one or two food attributes. An example is the macronutrient self-selection paradigm, in which rats are given three simple foods – that is, foods containing a single macronutrient (fat, protein, or carbohydrate; e.g., Shor-Posner et al., 1991). Other techniques include adding nonnutritive flavorings to water or a standardized diet and using nonnutritive bulk to reduce caloric density while preserving the macronutrient profile (e.g., Galef & Whiskin, 1995; Johnson & Collier, 2001; Naim et al., 1986). This reductionistic strategy places a premium on internal validity, which strengthens causal inferences about specific food attributes; it has been and remains an invaluable approach. That strategy, however, can limit external validity. For instance, intermittent access to fat affects calorie intake differently depending on whether a pure fat or a complex high-fat food is used (Davis et al., 2007), and rats sometimes respond differently to a tastant in water versus food (e.g., Dess, Madkins, et al., 2013; Mook, 1974; Wong, 1985).

Moreover, outside of the laboratory, food attributes tend to be “naturally confounded” (e.g., high-fat foods are calorically dense, sugary foods taste sweet), and their form, availability, and appeal are tied to an animal’s evolutionary and life history (Diehl, 2003; Román-Palacios et al., 2019; Rosati, 2017). The reductionistic tradition is not well suited to addressing how the whole may be more than the sum of parts. Synergistic effects of fat, sugar, and caloric density illustrate the limitations of a highly analytical approach and the hunt for a single dietary “culprit” such as fat or sugar (Hoch et al., 2014; Ramirez & Friedman, 1990). Furthermore, rats are often viewed in terms of a few characteristics shared with humans that make them a convenient, abstract “model organism” rather than a particular sort of creature. *Rattus norvegicus* is an opportunistic omnivore that typically lives in proximity to *Homo sapiens*, and, to a large extent, they eat foods that humans eat (Barnett, 1963, 2007, 2001; Modlinska & Pisula, 2020). Outside of the laboratory, their food landscape usually is complex and variable with respect to macronutrients, micronutrients, and sensory properties. Complementing the reductionistic tradition by providing a diverse array of foods to domesticated strains can help bridge the literatures on food intake in humans and in free-living and laboratory rats.

A classic study of free-living Norway rats documented their consumption of grains, sweetened food, cabbage, and horse liver (Barnett & Spencer, 1953). Since then, relatively few studies have focused on laboratory rats’ intake of the foods that their cousins find in pantries, dumpsters, and gardens. Foods such as potato chips, breakfast cereal, and salami are used in the cafeteria diet, junk food, and comfort food paradigms (Jarosz et al., 2006; Lanza & Snoeren, 2021; Ortolani et al., 2011). However, the focus usually is on total energy intake, meal and snacking patterns, and the consequences of consuming such diets. Perhaps setting a norm, the authors of a seminal cafeteria-diet study stated, “because of the complexity of the diet, food intake measures were not taken” (Sclafani & Springer, 1976, p. 462). Typically, when intake data are collected, measures are aggregated across foods and reported as total energy intake or macronutrient profiles (e.g., Gomez-Smith et al., 2016; Oliva et al., 2017).

In some cases, intake of individual foods is reported. For instance, Martire et al. (2013) provided rats with eight test foods and periodically recorded intake of four of the foods; rats consumed more calories from meat products and cake than from cookies. Shafat et al. (2009) reported the intake of 36 test foods with the highest intake (in grams and calories) for shredded wheat and the lowest for honey, which was rejected. In both studies, a variety of four snack foods was available each day, a procedure appropriate to providing variety and increasing energy intake but not to assessing intake of a particular food or choice. Blending junk foods into a mash (Lesser et al., 2017) exemplifies most cafeteria/junk/comfort food researchers’ interest in how high-energy palatable foods affect physiology and behavior. Consequently, little information is available on the intake of foods used in those paradigms.

The present study provides the most comprehensive systematic description to date of female and male laboratory rats’ intake of foods representative of the foods they encounter outside of the laboratory. The test foods balanced the internal validity concerns that drive the reductionistic strategy with greater ethological validity of the foods used. Some foods were grocery store products, with the selections including versions that were lower and higher in a target attribute (fat, sodium, cacao, etc.). Of course, those foods differ in more than one attribute. The lower and higher fat peanut butters, for instance, contained different amounts and kinds of sweeteners. Describing intake of such off-the-shelf foods has value, but multiple differences limit interpretation of results vis à vis any particular attribute. We, therefore, complemented those experiments with studies of custom foods that allowed stronger inferences about two attributes: proportion of calories from fat and caloric density.

The Occidental High- and Low-Saccharin-Consuming rat lines (respectively, HiS and LoS) were used to model intraspecies diversity. The line difference on the selection phenotype of voluntary saccharin intake arises from more avid intake of sweet tastants by HiS rats and greater sensitivity to bitter sidetastes among LoS rats (Dess, 2000; Dess & Chapman, 2020; Dess et al., 2017). The lines also differ (HiS > LoS)

on intake of starch, ethanol, and salt solutions (Dess, 2000; Dess et al., 1998; Dess et al., 2017). We had few predictions about line differences in the present study. In a simple world, the saccharin-intake phenotype reflects constraint-free reward sensitivity, such that HiS rats will eat more of any palatable food than LoS rats. Indeed, in one study, HiS rats ate more during intermittent access to a cookie mixture than LoS rats (Yakovenko et al., 2011).

However, such a simple world would require domain-general rewards to overwhelm the effects of flavors, calories, and macronutrient profile on food intake. We expected results for a diverse array of foods to be more complicated. In a pilot study in which chow, sucrose solution, and high-fat cookie were available (described in Dess, 2001), HiS rats took calories indiscriminately from all three sources whereas LoS rats were choosier, taking more calories from the cookie than from the sucrose; both lines took about a third of their calories from chow. Furthermore, relative to HiS rats, LoS rats show less elastic operant responding for food (Dess et al., 2007) and greater sensitivity to negative energy balance (Dess et al., 2000; Dess, Chapman, et al., 2013; McLaughlin et al., 2011; VanderWeele et al., 2002). Based on their greater responsiveness to nutritional status, LoS rats might be expected to be more discerning of at least some food attributes.

## General Method

### Rats

Adult rats (60-90 days of age) from the outbred Occidental College High- and Low-Saccharin Consuming rat lines were used in all experiments. Females (~270 g) and males (~400 g) were used in all experiments except Experiment 5B (males only). Most group sizes were  $n = 11-12$  (range = 10-16). At least five litters per line were represented in each experiment.

Rats were individually housed in hanging stainless steel cages during data collection, in a temperature-controlled room (22.5°C) on a 12:12 light:dark cycle with lights on at 7:00 a.m.. Purina 5001 Rodent Chow and water were continuously available. Care and use of the rats adhered to ILAR's *Guide for Care and Use of Laboratory Animals* (2011) and a protocol approved by the Occidental College Institutional Animal Care and Use Committee.

### Data Collection Procedures

#### *Baseline Measures*

After several days of adaptation to individual housing, water intake (24 hr) and chow intake (overnight, to match the test-food period) were measured for two days, and the rats were weighed.

#### *Food Tests*

After baseline measurements, a series of overnight food tests was conducted. Weighed portions of test foods were presented in glass jars in stainless-steel holders between 3:00 and 4:00 p.m.. Portions were based on pilot data to ensure they were large enough to prevent rats from running out. A portion of ~50 g of each test food was presented in a 4 oz jar with three exceptions: In Experiment 5, portions of each chocolate were ~30 g; in Experiment 6, rats were given two 4 oz jars, each containing ~70 g in fruit tests and ~140 g in vegetable tests; and in Experiment 7, portions of ~35 g of each macronutrient were presented in 2.5 oz jars. Uneaten test food (including spillage) was collected and weighed the next morning between 8:00 and 9:00 a.m.. Tests that are 17-18 hr long are relatively insensitive to neophobia and to strain differences in neophobia in free-living and laboratory rats (Modlinska et al., 2015), and any such effects were minimized by balancing test orders.

All test food intake was voluntary in the sense that chow was continuously available. Chow intake during the test-food period was recorded. Only chow and water were available between collection of uneaten test food in the morning and the next test that afternoon, as a washout period. Perishable foods were refrigerated before use.

## Research Designs

Between-group factors were line (LoS, HiS) and, with one exception (Experiment 5B), sex (female, male). Repeated-measures designs were used for food tests. Specifically, a Latin square was used to balance the order in which foods were tested. Littermates were balanced across test orders. Two test sequences were used. In *successive* tests, rats had one test food at a time. In *choice* tests, rats had two test foods at a time. Details specific to each experiment are described below.

## Statistical Analyses

Univariate analyses of variance (ANOVA) were used to evaluate the comparability of groups on baseline measures and on test food intake. All test statistics with  $p \leq .05$  were considered significant and are reported in the text.

**Analysis of Baseline Comparability.** Each of the three baseline measures was subjected to a Line  $\times$  Sex analysis of variance (ANOVA). A total of 34 comparisons in 12 experiments (water and chow intake not measured in Experiment 5B) yielded eight line differences. LoS rats drank  $\sim 5$  g more than did HiS rats in Experiment 1B [ $F(1, 40) = 11.56, p = .002$ ], and HiS rats weighed  $\sim 36$  g more than LoS rats in Experiment 5A [ $F(1, 45) = 7.55, p = .01$ ]. Neither difference is characteristic of the lines, and, as explained below, the line difference in chocolate intake in Experiment 5 was not an artifact of body weight. Chow intake was greater among LoS rats than HiS rats in Experiments 1A [ $F(1, 45) = 13.35, p < .001$ ], 1B [ $F(1, 41) = 5.85, p = .02$ ], 2A [ $F(1, 51) = 7.69, p = .008$ ], 5A [ $F(1, 45) = 16.61, p < .001$ ], and 6 [ $F(1, 43) = 6.22, p = .02$ ], with an average difference of 2.7 g (range = 1.9-3.4); in Experiment 7, chow intake was 3.6 g greater among LoS males than HiS males [Line  $\times$  Sex interaction,  $F(1, 41) = 9.50, p = .004$ ]. We sometimes have seen a tendency toward greater food intake by LoS rats (e.g., Dess et al., 2007; Dess et al., 2018), which could arise from line differences in metabolic efficiency and/or gut microbial communities (Dess, Chapman, et al., 2013; Dess et al., 2020; Lyte et al., 2016) and could be related to dietary preferences. The pattern of results in the present study, however, was not a byproduct of one line generally drinking, eating, or weighing more or less: Line differences in test-food intake ran in both directions, were observed regardless of whether baseline measures differed, and were specific to certain types of foods. Inconsistent line differences in baseline measures do not compromise interpretation of these results.

**Analysis of Food Test Data.** Each experiment reported below has a “Results and Discussion” section in which results are described and interpreted and a statistical analysis is provided, as follows: Data from successive and choice tests were subjected to separate Line  $\times$  Sex  $\times$  Food Kind(s) mixed-design ANOVAs; food kinds varied across experiments. Where the sphericity assumption for repeated measures was violated (Mauchly’s test  $p \leq .05$ ), Greenhouse-Geisser (GG) corrected  $p$  values are reported. For each variable, the highest order interaction involving the variable was interpreted with pairwise contrasts, using the Bonferroni correction for Type I error. Contrasts on means from successive tests compared groups (based on line and/or sex) at each level of the other variables. Contrasts on means from choice tests compared intake of the two test foods at each level of the other variables.

In light of the number of experiments, groups, and conditions, significant results were made visually accessible in graphs by averaging across levels of variables that had no significant main effect or interaction with other variables. In lieu of graphical representation, means (with standard errors, SEMs) are reported in the text when only one main effect was significant (marginal means reported) or when no effects or interactions were significant (grand mean reported). For Experiments 1-5, graphs are scaled to a maximum of 40 g to facilitate comparisons across experiments. Different scales were used in Experiments 6 and 7 to make significant differences visible.

## Experiments 1A-1B: Grocery Store Foods

Rats were tested with lower-fat and higher-fat fat versions of three grocery store foods: peanut butter, cream cheese, and cookies. These foods were chosen due to their contrasting macronutrient profiles (respectively, balanced, high protein/low carbohydrate, high carbohydrate/low protein). In Experiment 1A, rats were tested with all six foods, one at a time. In Experiment 1B, rats were given three choice tests, with the low- and high-fat versions of one kind of food available at the same time.

## Experiment 1A: Grocery Store Foods – Successive Tests

### Materials and Procedure

Each rat was tested once with low- and high-fat versions of peanut butter (reduced-fat and regular Jif, J. M. Smucker Co.), cookie (Signature Select Animal Crackers and Keebler Sandies Classic Shortbread, pulverized), and cream cheese (Kroger Fat-Free and Original, whipped). The caloric density and fat content of each food are shown in Table 1A. Tests were conducted on consecutive days. The Latin square that was used to balance testing order was designed with three constraints: No rat got two high-fat versions or the same type of food in a row, and whether the first test food was the low-fat version or the high-fat version was balanced

**Table 1**

### Caloric Density and Proportion of Calories from Fat

A. Grocery store versions of foods in experiments 1A-1B						
	Peanut Butter		Cheese		Cookies	
	Caloric Density	Prop. Cal. From Fat	Caloric Density	Prop. Cal. From Fat	Caloric Density	Prop. Cal. From Fat
Low Fat	5.28	0.57	1.07	0	4.00	0.15
High Fat	5.76	0.76	3.21	0.90	5.42	0.54
B. Custom versions of foods in experiments 2A-2C						
	Peanut Butter		Cheese		Cookies	
	Caloric Density	Prop. Cal. From Fat	Caloric Density	Prop. Cal. From Fat	Caloric Density	Prop. Cal. From Fat
Base	3.24	0.61	2.04	0.76	2.60	0.50
High Calorie	4.75	0.61	3.68	0.76	4.80	0.50
Low Fat	3.24	0.36	2.04	0.24	2.60	0.27
C. Grocery-store and custom versions of Spam in experiments 4A-4B						
	Grocery Store Spam			Custom Spam		
Type	Caloric Density	Prop. Cal. From Fat	Type	Caloric Density	Prop. Cal. From Fat	
Classic	3.21	0.80	Base	2.85	0.78	
Reduced Sodium	3.21	0.80	High Calorie	4.67	0.78	
Lite	1.96	0.65	Low Fat	2.85	0.51	

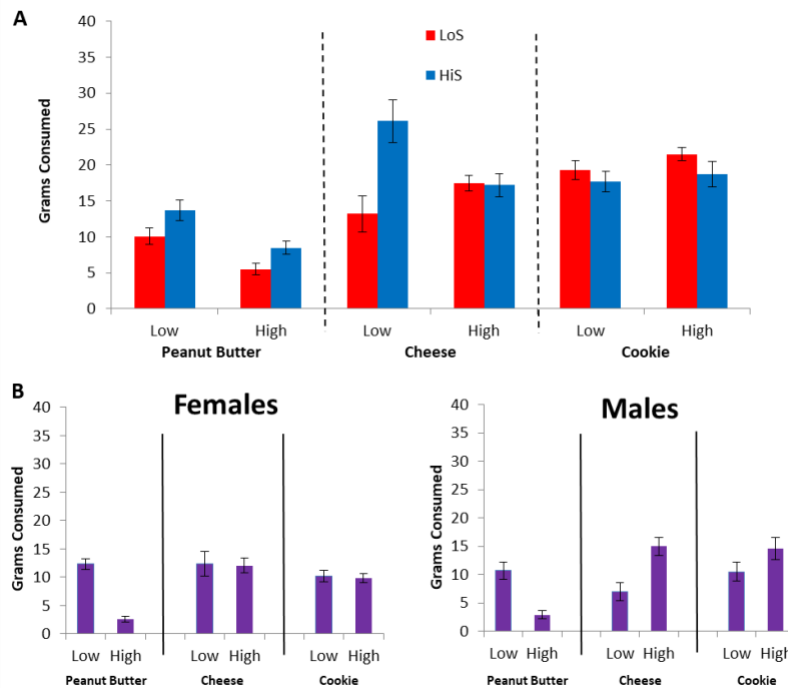
## Results and Discussion

Figure 1A shows intake of each test food. Rats ate less peanut butter than cheese or cookie. Although each version of peanut butter was higher in calories and fat than its counterpart in the other types of food, postingestive feedback does not account for lower consumption of peanut butter. The calories and fat in the low-fat peanut butter were similar to the high-fat cookies, yet rats ate more of the latter. These facts implicate other attributes (e.g., carbohydrate/protein ratio) and/or sensory cues for those attributes in lower peanut butter intake.

With respect to fat levels, rats ate similar amounts of the low-fat and high-fat cheese and cookie but more low-fat than high-fat peanut butter. The peanut butter finding is consistent with postingestive effects limiting intake of higher fat foods. Here, however, any such effect occurred only for the food with the most balanced macronutrient profile.

### Figure 1

#### Intake of Grocery-Store Foods



Note. Means and SEMs are shown. “Low” = lower fat version; “High” = higher fat version. Panel A (upper), successive tests (Experiment 1A); Panel B (lower), choice tests (Experiment 1B).

Interestingly, HiS and LoS rats did not differ on intake of low- or high-fat cookie, the foods with the highest percentage of calories from sugar (18% and 20%) and total carbohydrate (50% and 83%) (cf. the low-fat and high-fat peanut butters at, respectively, 8% and 6% sugar and 32% and 17% total carbohydrate). The saccharin phenotype, then, is not a reliable predictor of intake of everything sweet and/or high in carbohydrate. HiS and LoS rats did differ on intake of low-fat cheese and both peanut butters, with HiS rats eating more of those foods than LoS rats. Whatever mechanism accounts for greater intake of those foods among HiS rats, it does not distinguish the lines when high-fat cheese or cookies are available.

A Line  $\times$  Sex  $\times$  Food Type (peanut butter, cheese, or cookie)  $\times$  Fat Level (low or high) ANOVA yielded main effects of food type [ $F(2, 90) = 57.90, p < .001$ ] and fat level [ $F(1, 45) = 4.60, p = .04$ ] and the following interactions: Food Type  $\times$  Fat Level [ $F(2, 90) = 8.84$ , GG-corrected  $p < .001$ ], Line  $\times$  Food Type [ $F(2, 90) = 8.97, p < .001$ ], Line  $\times$  Fat Level [ $F(1, 45) = 8.36, p = .01$ ], and Line  $\times$  Food Type  $\times$  Fat Level [ $F(2, 90) = 10.81$ , GG-corrected  $p < .001$ ]. Contrasts on food types yielded the following: cheese = cookie  $>$  peanut butter. Contrasts comparing the low-fat and high-fat version of each type of food yielded a difference only for peanut butter. Contrasts comparing HiS and LoS rats on each of the six test foods yielded a line difference (HiS  $>$  LoS) for low-fat cheese, low-fat peanut butter, and high-fat peanut butter.

## **Experiment 1B: Grocery Store Food – Choice Tests**

### ***Materials and Procedure***

The same foods as in Experiment 1A were used. Rats were given three choice tests on consecutive days. In each test, the low-fat and high-fat version of a food (peanut butter, cheese, or cookies) were simultaneously available. A Latin square design was used to balance test order.

### ***Results and Discussion***

Figure 1B shows intake of test foods in the three choice tests. Several results were the same as in the successive tests: Rats ate less peanut butter than cheese or cookie, ate more low-fat than high-fat peanut butter, and ate similar amounts of the two cookie versions. In striking contrast to the successive tests, rats ate more high-fat cheese than low-fat cheese, a preference expressed primarily by males. Notwithstanding the overall preference for high-fat cheese, female rats (but not males) ate more of low-fat foods than high-fat foods overall, a small preference primarily due to peanut butter choice. No line differences were observed.

A Line  $\times$  Sex  $\times$  Food Type (peanut butter, cheese, or cookie)  $\times$  Fat Level (low vs. high) ANOVA yielded a main effect of food type [ $F(2, 82) = 15.11$ , GG-corrected  $p < .001$ ] and two-way interactions of fat level with food type and with sex [respectively,  $F(2, 82) = 22.01$ , GG-corrected  $p < .001$ , and  $F(1,41) = 8.20, p = .01$ ]. Contrasts showed lower intake of peanut butter than the other foods and two fat-level differences: low-fat  $>$  high-fat peanut butter, and high-fat  $>$  low-fat cheese. Contrasts between fat levels separately for females and males showed that overall low-fat food intake exceeded overall high-fat food intake only among the females. Although the Food Type  $\times$  Fat Level  $\times$  Sex interaction was not significant, Figure 1B plainly shows that males drove the preference for high-fat cheese and that peanut butter accounts for the female rats' greater intake of low-fat than high-fat foods. This complicated pattern illustrates that even when higher-order interactions are not significant, generalizing across types of food should be done with caution.

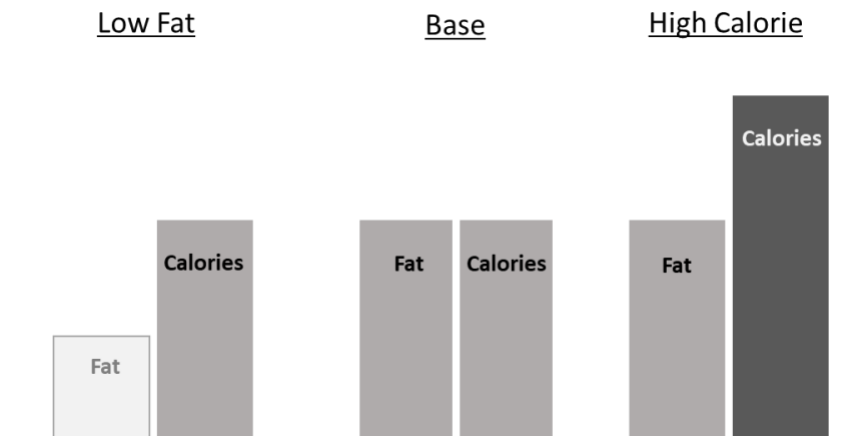
## Experiments 2A-2C: Custom Foods

In Experiments 2A-2C, rats were tested with custom versions of, respectively, peanut butter, cheese, and cookie. We customized these foods to control for caloric density and proportion of calories from fat to explore the role of those attributes in intake and choice. Three custom versions of each type of food were created using nonnutritive ingredients (cellulose, mineral oil) and nutritive ingredients tailored to the corresponding grocery store food; for example, sources of calories were animal-based protein (casein) and fat (lard) in Spam versions and a carbohydrate (corn starch) and plant-based fat (soybean oil) in cookie versions. A “base” version was a reference food to which the other versions could be compared. The low-fat version had a lower proportion of calories from fat than did the base version but the same caloric density. The high-calorie version had a higher caloric density than did the base version but the same proportion of calories from fat. The amounts of nonnutritive ingredients in the base version were sufficient to allow reducing the proportion of calories from fat without reducing calorie density (low-fat version) and increasing caloric density without increasing the proportion of calories from fat (high-calorie version).

Figure 2 is a graphic representation of how the base version related to the other versions, and Tables 1B and 1C show the caloric density and proportion of calories from fat for Experiments 2 and 4. Recipes for all custom foods are available on request. It was not possible to match caloric density or fat content of foods across experiments while producing foods for each experiment with acceptably similar textures. Instead, for each experiment, custom foods were formulated such that the low-fat version had no more than 40% of the fat content of the base version and the high-calorie version had at least 40% more calories per gram than the base version. In each experiment, each rat was tested with base, low-fat, and high-calorie versions of one kind of food (peanut butter, cheese, or cookie) in two ways. First, the versions were tested one at a time, with test order balanced (successive tests). Then, after one day off, each rat was given two choice tests, in each of which the base version and one alternative version were available simultaneously (base/low-fat test, base/high-calorie test); test order was balanced.

**Figure 2**

*Depiction of the Base Version Relative to the Low-Fat and High-Calorie Versions*



*Note.* Different densities of shading are used to convey distinctively low fat content in the Low Fat foods (vs Base and High Calorie, which are the same) and distinctively high caloric density in the High Calorie foods (vs Base and Low Fat, which are the same).

## Experiment 2A: Custom Peanut Butter

### Materials

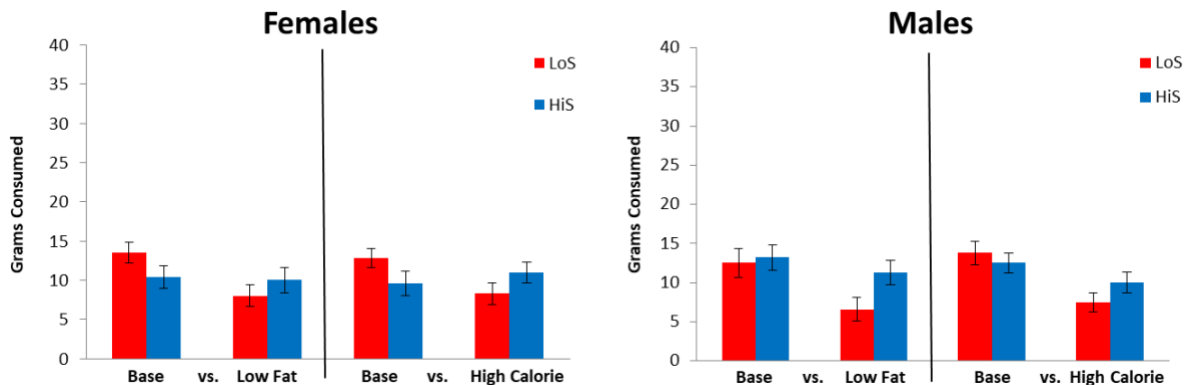
For the base, low-fat, and high-calorie versions of peanut butter, nutritive ingredients were 100% pure peanut powder (Crazy Richard's), soybean oil (CVS Pharmacy Gold Emblem Vegetable Oil), and glucose (Millipore/Sigma Aldrich). The nonnutritive ingredients were mineral oil (CVS Pharmacy) and cellulose (Alphacel, MP Biomedicals).

### Results and Discussion

In the successive tests, rats ate about the same amount of all three versions of peanut butter (grand mean  $18.1 \pm 0.6$  g), and no line differences were observed. These results suggest that fat content and caloric density are not strongly determinative of peanut butter intake; other attributes such as flavor or mouthfeel arising from specific ingredients (e.g., type and amount of sweetener) must account for differences in peanut butter intake in Experiment 1A (Oliva et al., 2017). Male rats ate more peanut butter than did female rats (respectively,  $19.4 \pm 0.8$  g vs.  $16.7 \pm 0.9$  g). A Line  $\times$  Sex  $\times$  Peanut Butter Version ANOVA yielded only a main effect of sex [ $F(1, 50) = 4.95, p = .03$ ].

**Figure 3**

*Intake of Custom Peanut Butter in Choice Tests (Experiment 2A)*



Note. Means and SEMs are shown.

Figure 3 shows intake of the base version and the alternative concurrently available (low fat or high calorie) in the two choice tests. Overall, rats ate more of the base version than the alternatives. HiS and LoS rats differed in the choice tests in two ways. First, LoS rats were more discriminating than were HiS rats. LoS rats ate more of the base version regardless of which alternative was available, whereas HiS rats ate about the same amount of base and alternative versions. A parsimonious explanation of greater intake of the base version by LoS rats – that it tasted better to them – begs the question of why it would taste better to them and not HiS rats. Notably, LoS rats only ate different amounts of the peanut butter versions when they had a choice between versions (i.e., not in the successive tests), an illustration of the limitations to inferring palatability, discriminability, or other constructs from amounts consumed in a single test procedure.

Second, when base and low-fat peanut butters were available, HiS males ate more peanut butter than did LoS males. In contrast to the LoS rats' choosiness, the heightened avidity of HiS males in the base/low-fat choice test was indiscriminate; they ate equal amounts of both versions, as they had in the successive tests. No such line difference in avidity was observed among females: HiS females were as indiscriminate as were HiS males, but their total peanut butter intake did not differ from LoS females and did not depend on which alternative was available.

A Line  $\times$  Sex  $\times$  Peanut Butter Choices (base vs. alternative)  $\times$  Alternative (low-fat or high-calorie alternative) ANOVA yielded a main effect of peanut butter choices [ $F(1, 50) = 7.66, p = .008$ ] and three interactions, all involving line: Line  $\times$  Peanut Butter Choices, Line  $\times$  Sex, and Line  $\times$  Sex  $\times$  Alternative [respectively,  $F(1, 50) = 4.15$  with  $p = .05$ ,  $7.62$  with  $p = .008$ , and  $4.64$  with  $p = .04$ ]. Contrasts confirmed that only LoS rats ate significantly more of the base than the alternatives and that total intake in base/low-fat test was greater among HiS males than LoS males.

## Experiment 2B: Custom Cream Cheese

### Materials

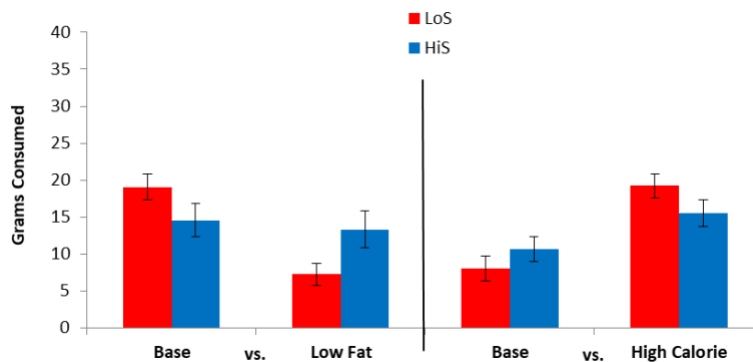
For the base, low-fat, and high-calorie versions of cheese, the nutritive ingredients were fat-free cream cheese (Kroger Fat Free), casein (MP Biomedicals), locust bean gum (Modernist Pantry), kappa carrageenan (Modernist Pantry), and soybean oil (CVS Pharmacy Gold Emblem Vegetable Oil). The nonnutritive ingredients were cellulose (Alphacel, MP Biomedicals) and mineral oil (CVS Pharmacy).

### Results and Discussion

In the successive tests, cheese intake (grand mean  $19.9 \pm 0.9$  g) did not differ between groups or cheese versions. A Line  $\times$  Sex  $\times$  Cheese Version ANOVA yielded no main effects or interactions. Figure 4 shows intake of the base version and the alternative version of cheese (low fat or high calorie) concurrently available in the two choice tests. As with peanut butter, LoS rats were more discriminating than HiS rats. However, unlike results for peanut butter, their intake of the base version relative to the alternative depended on which alternative was available: They ate more of the base version than a lower-fat alternative, and less of the base version than a higher-calorie alternative. HiS rats showed the latter preference but to a lesser degree.

**Figure 4**

*Intake of Custom Cheese in Choice Tests (Experiment 2B)*



Note. Means and SEMs are shown.

A Line  $\times$  Sex  $\times$  Cheese Choices (base vs. alternative)  $\times$  Alternative Type (low-fat or high-calorie) ANOVA yielded two interactions: Cheese Choices  $\times$  Alternative Type and Line  $\times$  Cheese Choices  $\times$  Alternative Type [respectively,  $F(1, 44) = 49.40$  and  $16.58$ ,  $ps < .001$ ]. Contrasts showed that LoS rats ate more of one version than the other in both tests, whereas HiS rats only ate more high-fat than base cheese.

An emerging pattern in this series of experiments seems to be greater choosiness among LoS rats, with the preferences depending on the kind of food. As for HiS rats' avidity for peanut butter in one choice test (base/low-fat; Experiment 2A), no such tendency manifested with cheese. The experiments below allowed us to evaluate the consistency of these patterns across other complex foods.

## Experiment 2C: Custom Cookie

### Materials

For the base, low-fat, and high-calorie versions of powdered cookie, the nutritive ingredients were shortbread cookie (Keebler Sandies Classic Shortbread), cornstarch (Signature Select Pure), soybean oil, (CVS Pharmacy Gold Emblem Vegetable Oil) and glucose (Millipore/Sigma Aldrich Inc.). The nonnutritive ingredients were cellulose (Alphacel, MP Biomedicals) and mineral oil (CVS Pharmacy).

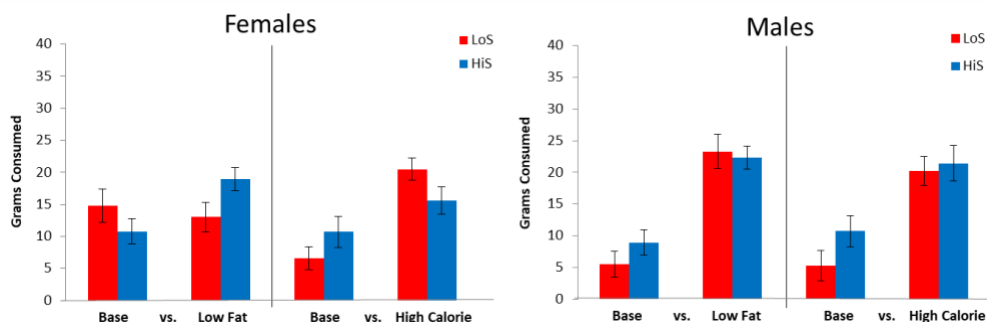
### Results and Discussion

In the successive tests, cookie intake (grand mean  $21.2 \pm 1.2$  g) did not differ between groups or cookie versions. A Line  $\times$  Sex  $\times$  Cookie Version ANOVA yielded no significant main effects or interactions. Thus, in successive tests with all three custom foods (peanut butter, cheese, and cookie), intake was not influenced by caloric density or fat content and did not differ between LoS and HiS rats.

Figure 5 shows intake of the base version and the alternative (either low-fat or high-calorie) in the two choice tests. Although intake of some experimental high-carbohydrate foods depends on caloric density and not fat (Ramirez & Friedman, 1990), intake of this high-carbohydrate food was influenced by both caloric density and fat content: Rats ate less of the base version than either the low-fat or the high-calorie version. In contrast to the indiscriminate intake when given a choice between lower and higher fat grocery store cookies (Experiment 1B), these preferences were quite robust. Two group differences were apparent. First, when a high-calorie alternative was available, HiS males ate more cookie than did LoS males. HiS and LoS females did not differ. Second, the preference for the alternatives over the base version was more pronounced among males than females.

**Figure 5**

*Intake of Custom Cookie in Choice Tests (Experiment 2C)*



*Note.* Means and SEMs are shown.

A Line  $\times$  Sex  $\times$  Cookie Choices (base vs. alternative)  $\times$  Alternative Type (low-fat or high-calorie) ANOVA yielded main effects of cookie choices and alternative type [respectively,  $F(1, 48) = 38.07$  with  $p < .001$  and  $4.04$  with  $p = .05$ ] and two interactions: Sex  $\times$  Cookie Choices and Line  $\times$  Sex  $\times$  Alternative Type [respectively,  $F(1, 48) = 5.64$  with  $p = 0.02$  and  $4.65$  with  $p = .04$ ]. Contrasts confirmed that HiS and LoS males (not females) differed on total cookie intake only on the base/low-fat test. Contrasts also yielded a base  $<$  alternative difference for both females and males but the difference was larger (with a smaller  $p$  value) in males.

### **Experiment 3: Matched Foods**

A custom version of peanut butter, of cheese, and of cookie was designed such that all three foods had the same caloric density and percentage of calories from fat. Matching the three foods on these attributes allowed us to examine how other attributes distinguishing the foods influence intake.

#### ***Materials and Procedure***

The nutritive and nonnutritive ingredients for each of the three foods were the same as described in Experiment 2A-2C. For all three foods, the caloric density was 2.8 cal/g, and the proportion of calories from fat was 57%. As in Experiment 2, rats received three successive tests (one test food at a time) and, after a day off, a series of choice tests. Three choice tests were conducted, with each food paired with each of the other foods. Test order was balanced using a Latin square design.

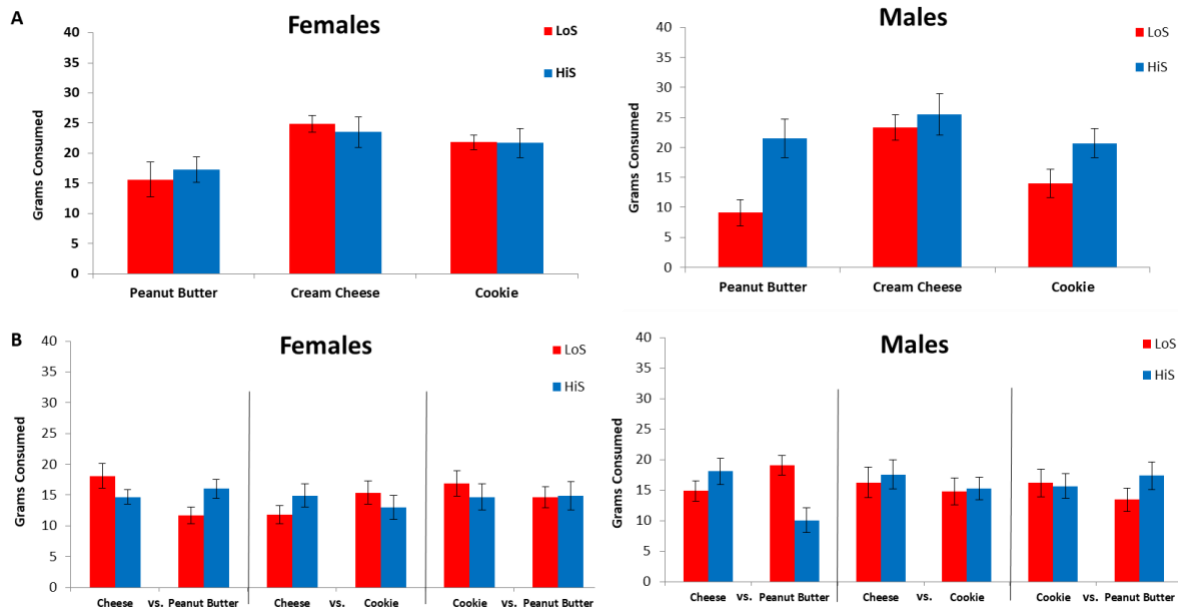
#### ***Results and Discussion***

Figure 6A shows intake of peanut butter, cheese, and cookie in the successive tests. Rats ate less peanut butter than cookie and less cookie than cheese. This result indicates that the lower intake of peanut butter in Experiment 1 was not an artifact of the sweeteners, caloric density, or fat content of the products used in Experiment 1 but rather had to do with attributes that distinguish peanut butter from cream cheese and cookies. As observed in custom peanut butter and cookie choice tests, HiS males ate the matched foods more avidly than did LoS males whereas HiS and LoS females did not differ.

A Line  $\times$  Sex  $\times$  Food Type ANOVA yielded main effects of line and food type [respectively,  $F(1, 46) = 4.34$  with  $p = .04$  and  $F(2, 92) = 16.26$  with  $p < .001$ ] and a Line  $\times$  Sex interaction [ $F(1, 46) = 4.28$ ,  $p = .04$ ]. Contrasts comparing each food to each of the other foods confirmed intake in the following order: peanut butter  $<$  cookie  $<$  cheese. Contrasts comparing HiS and LoS rats of each sex for overall test food intake showed a line difference only for males.

**Figure 6**

*Intake of Matched Custom Foods (Experiment 3)*



Note. Means and SEMs are shown. Panel A (upper), successive tests; Panel B (lower), choice tests.

Figure 6B shows intake in the choice tests. Intake of test foods did not deviate much from ~15 g, and group differences were modest. Males ate more than did females in the test without peanut butter (cheese/cookie). No preferences were observed in tests with cookie (cheese/cookie, cookie/peanut butter), but, in the cheese/peanut butter test, LoS females and HiS males preferred cheese to peanut butter. That preference is consistent with those groups' relative intake of the two foods in the successive tests. Although HiS females and LoS males had also eaten more cheese than peanut butter in the successive tests, they did not express a preference for cheese over peanut butter when given a choice. Why groups differ in the predictive relationship between successive and choice tests for these two foods remains to be determined.

Due to the round-robin nature of the choice tests, data from the three tests were analyzed separately. A Line  $\times$  Sex  $\times$  Food Choices ANOVA on the cheese versus peanut butter test yielded a main effect of food type and a Line  $\times$  Sex  $\times$  Food Choices interaction [respectively,  $F(1, 46) = 7.57$  with  $p = .008$  and  $6.16$  with  $p = .02$ ]. Contrasts confirmed greater intake of cheese than peanut butter only among LoS females and HiS males. ANOVAs on the other choice tests yielded only one significant effect: a main effect of sex in the cheese versus cookie test [ $F(1, 46) = 5.61$ ,  $p = .02$ ], with males eating more in that test than females.

In sum, matching peanut butter, cheese, and cookie on caloric density and fat content minimized preferences and group differences. The implication is that variation in caloric density and fat content does enhance preferences and group differences. However, the effects of those attributes depend on the type of food.

## Experiments 4A-4B: Grocery Store and Custom Spam

*Rattus norvegicus* is cannibalistic and a predator, aspects of evolutionary and life history crucial to understanding its bibehavioral systems. Yet meat consumption is understudied in laboratory strains. Meat is a source of amino acids that are precursors to neurotransmitters such as serotonin and dopamine, and meat consumption alters gut microbial diversity (see review by Lyte, 2019). Meat products have been included in cafeteria diets (Lalanza & Snoeren, 2021), but, to our knowledge, one- or two-food intake tests with meat products differing in specified attributes have not been conducted. Spam was selected for this experiment due to stability conferred by preservatives and the popularity of processed meat products. Experiment 4A examined three grocery-store versions of Spam, and Experiment 4B examined three custom versions of Spam.

### ***Materials and Procedure***

In Experiment 4A, rats were tested with three Spam products: Classic, Reduced Sodium (25% less sodium than Classic), and Lite (same sodium as Reduced Sodium, 50% less fat than Classic and Reduced Sodium). In Experiment 4B, three custom versions were used: base, low fat, and high calorie. Nutritive ingredients were Reduced Sodium Spam (Hormel), lard (Farmer John), and casein (MP Biomedicals). Nonnutritive ingredients were cellulose (Alphacel, MP Biomedicals) and mineral oil (CVS Pharmacy). Although sodium was not explicitly manipulated, we determined that the sodium content of the versions was base > high calorie > low fat, which was useful for purposes of comparing the results to the higher and lower sodium versions in Experiment 4A.

All Spam versions were blended into a paste in a food processor. Spillage weight was adjusted based on estimates of evaporation over the test period. No adjustment was made for evaporation from the jar, which was ~1 g for all varieties, so intake is overestimated by about a gram.

In both experiments, rats received three successive tests and, after a day off, two choice tests. In Experiment 4A, each choice was between Reduced Sodium and one of the other products. In Experiment 4B, each choice was between the base version and either the low-fat or the high-calorie version.

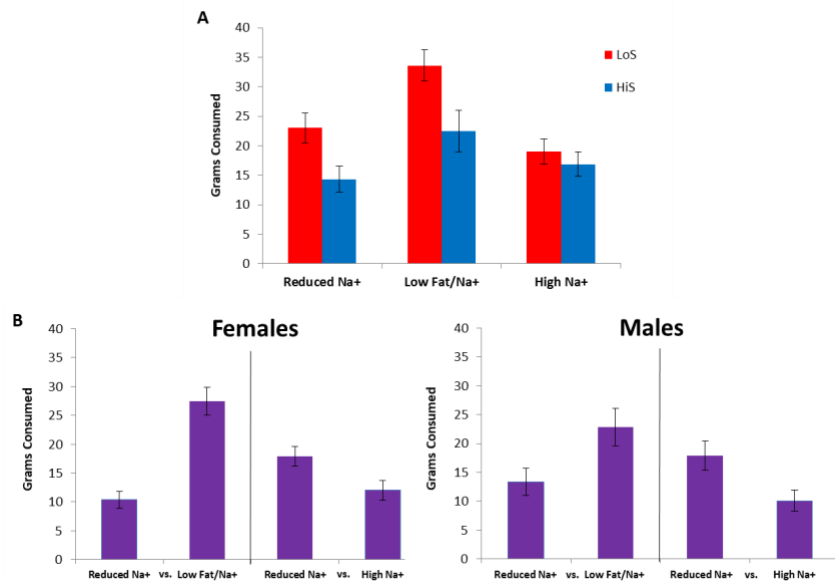
### ***Results and Discussion***

Figure 7A shows intake in the successive tests in Experiment 4A. Rats ate more Lite Spam (Low-Fat/Na+) than either Reduced Sodium (Reduced Na+) or Classic (High Na+) Spam. LoS rats ate more Spam than did HiS rats. Notably, LoS rats ate more Lite Spam (~35 g) than they had eaten of any other test foods in the preceding experiments. Spam is high in fat and protein and low in carbohydrate, so the avidity with which LoS rats ate it does not follow from the hypothesis that a 35% fat/65% carbohydrate optimally stimulates intake in satiated rats (Hoch et al., 2015); this finding suggests that such an optimal ratio pertains only to some kinds of food. A Line  $\times$  Sex  $\times$  Spam Type ANOVA yielded main effects of line and Spam type [respectively,  $F(1, 47) = 6.36, p = .02$ , and  $F(2, 94) = 19.53$ , GG-corrected  $p < .001$ ]. Contrasts confirmed that rats ate more Low-Fat/Na+ Spam than either of the other versions, intake of which did not differ.

Figure 7B shows intake in the choice tests in Experiment 4A. Rats ate less Reduced Sodium than Lite Spam (left panel) and ate more Reduced Sodium than Classic Spam (right panel). Interestingly, then, when given two Spam products, rats made healthy choices, preferring a lower to a higher fat alternative and a lower sodium to a higher sodium alternative. Although the latter preference is consistent with a report that rats preferred unsalted to salted solid food (Beauchamp & Bertino, 1985), rats will self-select a cafeteria diet that increases sodium intake (Oliva et al., 2017). Moreover, rats ate more Spam in the choice test with Reduced Sodium and Lite Spam, offsetting any reduction in sodium in those products. The present results do not point to “wisdom of the body” with respect to healthy eating. Whereas LoS rats had eaten more Spam than did HiS rats in the successive tests, the lines did not differ in the choice tests. Spam intake was similar in females and males, with a subtle difference: Females ate less of the Reduced Sodium Spam than the alternatives, an effect clearly attributable to Lite Spam. The same trend was apparent among males to a lesser extent.

**Figure 7**

*Intake of Grocery-Store Spam in Successive Tests (Experiment 4A)*



*Note.* Means and SEMs are shown. Panel A (upper), successive tests; Panel B (lower), choice tests.

A Line  $\times$  Sex  $\times$  Spam Choices (reduced Na+ vs. alternative)  $\times$  Alternative (low fat/Na+ or high Na+) yielded main effects of Spam choices and alternative [respectively,  $F(1, 47) = 7.96$  with  $p = .007$  and  $45.28$  with  $p < .001$ ] and two-way interactions of Spam choices with sex and with alternative [respectively,  $F(1, 47) = 4.46$  with  $p = .04$  and  $55.25$  with  $p < .001$ ]. Contrasts to interpret the Spam Choices  $\times$  Alternative interaction confirmed that intake of Reduced Sodium Spam was less than intake of Lite Spam and greater than intake of Classic Spam. Contrasts to interpret the Sex  $\times$  Spam Choices interaction showed that among females, intake of alternatives (averaged across tests) exceeded intake of the Reduced Sodium (averaged across tests); that difference was not significant among males. Although the Sex  $\times$  Spam Choices  $\times$  Alternative interaction was not significant, Figure 7B clearly shows that the two-way Sex  $\times$  Spam Choices interaction was driven by the females’ high intake of Lite Spam. This result reinforces a point made in Experiment 1B: Even when higher-order interactions are not significant, generalizing across foods should be done with caution.

The custom Spam versions used in Experiment 4B allowed determination of the replicability of the greater Spam intake by LoS rats in successive tests and of whether the preferences observed in the choice tests were due to fat and sodium or to other attributes that distinguished the grocery-store products. In the successive tests, LoS rats again ate more Spam than did HiS rats (respectively,  $21.8 \pm 1.1$  g vs.  $11.1 \pm 1.8$  g). Rats ate about the same amount of all three custom versions. A Line  $\times$  Sex  $\times$  Spam Type ANOVA yielded only a main effect of line [ $F(1, 37) = 26.87, p < .001$ ]. In the choice tests, Spam intake was similar across versions and groups. Rats in all groups tended to eat more of the base version ( $15.6 \pm 1.2$  g) than whatever the alternative was ( $12.2 \pm 1.2$  g), perhaps because the base version had the most Spam per 100 g. Rats also tended to eat more Spam in the base/low-fat test ( $14.7 \pm 0.9$  g) than in the base/high-calorie test ( $13.1 \pm 1.0$  g). However, both trends fell short of statistical significance. A Line  $\times$  Sex  $\times$  Spam Choices (base vs. alternative)  $\times$  Alternative (low fat or high calorie) ANOVA yielded marginally significant main effects of Spam choices [ $p = .06$ ] and alternative version [ $p = .10$ ].

The tendency for rats to eat more base than low-fat Spam in Experiment 4B suggests that the preference for Lite over Reduced Sodium Spam in Experiment 4A did not reflect a preference for less fat. Similarly, rats' tendency to eat more of the base version – which was highest in sodium – than either alternative suggests that the preference for Reduced Sodium over Classic Spam did not reflect a preference for less sodium. Other attributes of the grocery-store products such as kind of meat (pork/chicken mix) or flavorings appear to drive the preferences among the commercial products. When those attributes were controlled by using one Spam product (Reduced Sodium) to make all custom versions, LoS rats still ate more than do HiS rats when only one Spam version was available, but group differences in preference were virtually nonexistent. This pattern suggests that attributes common across Spam test foods – such as umami or “meatiness” – stimulate greater intake among LoS rats whereas attributes that distinguish the commercial products drive preferences.

### **Experiments 5A-5B: Chocolate**

In Experiment 5A, rats received a series of successive tests with four kinds of chocolate chips with increasing amounts of cacao: white, milk, semi-sweet, and dark. After a day off, the rats were given a choice between the two chocolate types eaten most avidly in the successive tests: white and milk chocolate. Chocolate is a simpler food than most of the foods in this series, and we had two predictions: Chocolate intake would decrease with increasing cacao because it is bitter, and HiS rats would eat more candy than would LoS rats. If the former prediction holds, one further might expect the line difference to grow larger as cacao increases due to LoS rats' greater sensitivity to bitter sidetastes.

Experiment 5B was a replication of the white versus milk chocolate choice test. The data come from two unpublished studies on how palatable food affects noningestive behaviors. They began identically, with presentation of pre-weighed white and milk chocolate chips mixed in one jar to male LoS and HiS rats. Data from the first chocolate exposure are directly comparable to the choice test in Experiment 5A and so are presented here.

#### ***Materials and Procedure***

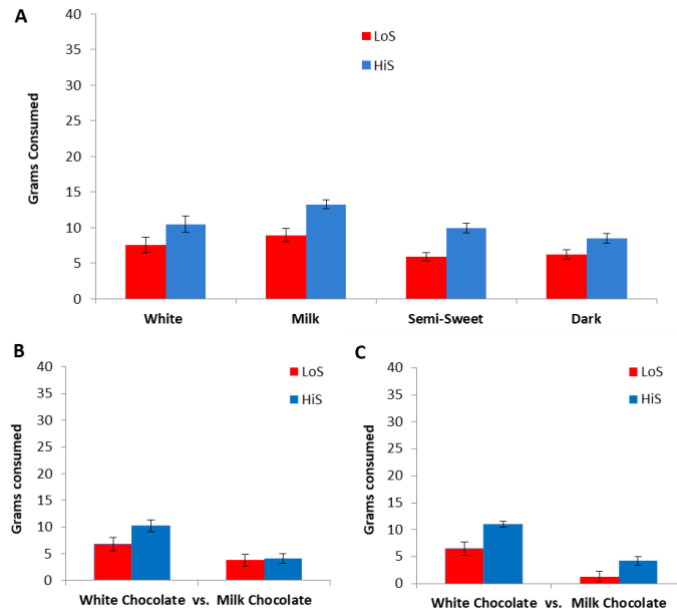
Four varieties of Nestlé chocolate chips were used in Experiment 5A: Premium White, Milk Chocolate, Semi-sweet, and Dark. These chips are similar in terms of caloric density (white, milk, and semi-sweet, 5.0 cal/g; dark, 5.7 cal/g) and fat content (45-56% calories from fat) but differ in amount of cacao (from 0% for white to 53% for dark), which is bitter. In Experiment 5A, successive and choice tests were conducted with a day off in between. In Experiment 5B, one choice test with Premium White and Milk Chocolate chips was conducted.

## Results and Discussion

Figure 8A shows chocolate intake in the successive tests in Experiment 5A. Rats ate more white and milk chocolate than semi-sweet or dark chocolate, and HiS rats ate more chocolate than did LoS rats. The line difference did not grow with cacao content, so these results provide no evidence of a role for cacao bitterness in the line difference. A Line  $\times$  Sex  $\times$  Chocolate Type ANOVA yielded main effects of line and chocolate type [respectively,  $F(1, 45) = 26.86, p < .001$  and  $F(3, 135) = 8.87, GG$ -corrected  $p < .001$ ] and a Line  $\times$  Sex interaction [ $F(1, 45) = 4.24, p = .04$ ]. Contrasts comparing each type to each other type yielded the following: white = milk > semi-sweet = dark. As for the Line  $\times$  Sex interaction, HiS rats in each sex ate more chocolate than did their LoS counterparts; the line difference was somewhat larger in males (with a smaller  $p$  value). Because this interaction was ordinal, means are not disaggregated by sex in Figure 8A.

**Figure 8**

*Intake of Chocolate (Experiments 5A-5B)*



*Note.* Means and SEMs are shown. Panel A (upper), successive tests; Panel B (lower left), choice test in Experiment 5A. Panel C (lower right), choice test in Experiment 5B.

In the choice test (Figure 8B), HiS rats ate more chocolate than did LoS rats. In addition, rats ate more white than milk chocolate and, unlike in the successive tests, chocolate intake did not differ between females and males. These results reinforce the value of conducting both successive (acceptability) and choice (preference) tests. Rats had eaten slightly less white than milk chocolate in the successive tests and yet, when given a direct choice, they strongly preferred white over milk chocolate. How much of a food is consumed when no tasty alternative is available is not a reliable proxy for preference of that food when two tasty foods are available. A Line  $\times$  Sex  $\times$  Chocolate Choices ANOVA yielded main effects of line and chocolate choices [respectively,  $F(1, 45) = 8.53$  with  $p = .005$  and  $9.85$  with  $p = .003$ ].

Figure 8C shows the intake of white and milk chocolate in Experiment 5B. As in the choice test in Experiment 5A, rats ate more white than milk chocolate, and HiS rats ate chocolate more avidly than did LoS rats. A Line  $\times$  Chocolate Choices ANOVA yielded main effects of line and chocolate choices [respectively,  $F(1, 31) = 19.56$  and  $39.83$ ,  $p < .001$ ]. This experiment replicates the results of Experiment 5A and shows that neither the line difference nor the preference for white over milk chocolate depends on prior experience with chocolates.

As noted in the General Method, HiS rats were heavier than were LoS rats in Experiment 5A. Secondary analyses show that the body weight difference does not account for the line difference in chocolate intake. First, the male HiS and LoS rats in Experiment 5B had virtually identical body weights (384.0 g and 384.1 g, respectively) yet the lines still differed in chocolate intake. Second, analyzing chocolate intake in Experiment 5A with body weight as a covariate (standardized within sex) did show that heavier rats ate more chocolate [covariate  $F(1, 44) = 18.99$ ,  $p < .001$ ] but, after controlling for body weight, the line difference was still significant [ $F(1, 44) = 15.72$ ,  $p < .001$ ; adjusted means of 7.6 g for LoS, 10.0 g for HiS].

### **Experiment 6: Fruits and Vegetables**

Two fruits and two vegetables were sampled from this diverse food group. In lieu of creating custom versions, we selected two baby food products in each category that differed in overall nutritional value (caloric density, nutrient richness). The lower and higher nutrition foods were, respectively, pear versus banana for fruits and pea versus sweet potato for vegetables.

#### ***Materials and Procedure***

Four Gerber Sitter 2<sup>nd</sup> Foods (pear, banana, pea, and sweet potato) were used. These products have minimal ingredients other than the fruit or vegetable. The manufacturer adds water to the pea and sweet potato products and, to prevent discoloration, adds ascorbic acid to the pear, banana, and sweet potato products and citric acid to the banana product. LoS and HiS rats do not differ in their response to citric acid (Dess, 2000), so the small amount of acid added to these foods would not be expected to differentially affect intake in the two lines.

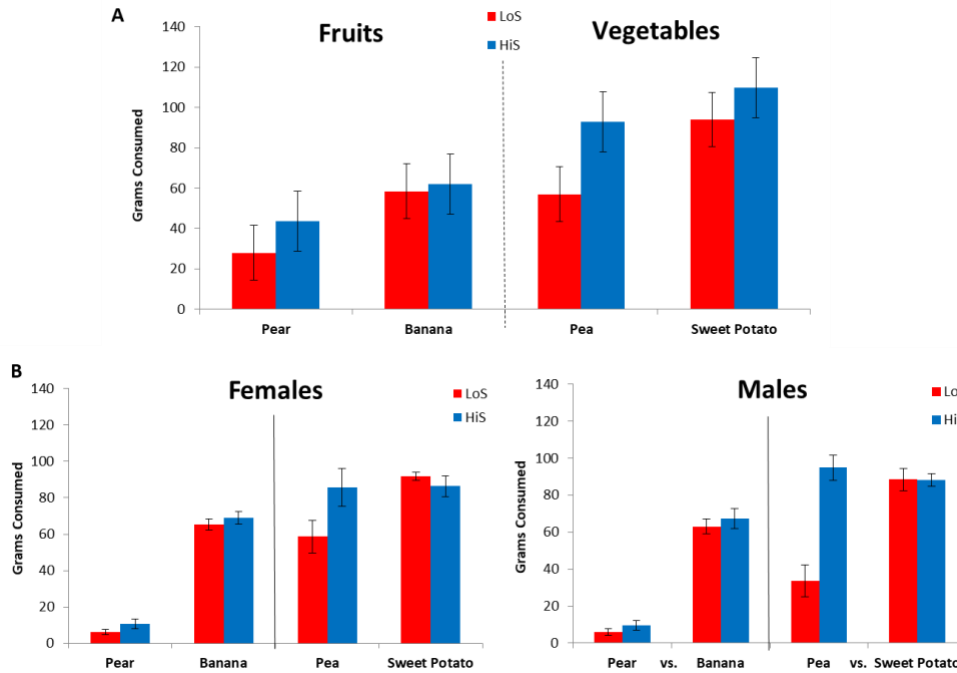
Each rat was tested with all four foods, with order balanced using a Latin square design. After a day off, each rat received two choice tests, one with fruits and one with vegetables; test order was balanced.

#### ***Results and Discussion***

Figure 9A shows intake in the successive tests. Rats ate more vegetables than fruits and ate more of the higher-nutrition vegetable and fruit than the lower-nutrition products. The overall rank ordering of the four foods is not explicable in terms of caloric density, sweetness, or protein content. For instance, the most avidly consumed food (sweet potato) had the same caloric density (0.6 cal/g) and less sugar (8% wt/wt) as the least avidly consumed food (pear; 11% sugar) and half the protein of the lower-nutrition vegetable (pea). Greater consumption of vegetables than fruits likely stems from the higher complex carbohydrate content of the vegetables (44%-71% of total carbohydrate, versus 17%-29% for fruits) for which starchy taste is a cue. Within a food type, on the other hand, caloric density, sugar or starch content, micronutrients, psychoactive compounds, and flavors could account for the greater intake of the food with higher total nutritional value. HiS rats ate more fruits and vegetables than did LoS. The line difference was larger for vegetables and for lower-nutrition foods. Also, females ate more fruits and vegetables ( $75.9 \pm 3.7$  g) than did males ( $61.8 \pm 4.2$  g).

**Figure 9**

*Intake of Fruits and Vegetables (Experiment 6)*



Note. Means and SEMs are shown. Panel A (upper), successive tests; Panel B (lower), choice tests.

These results show that both LoS and HiS rats are sensitive to the type and quality of these foods, eating more of the starchy vegetables than the fruits and eating more of the higher-nutrition food in each category. HiS rats ate these foods more avidly than did LoS rats, but that difference was smaller when the foods were higher in nutrition. The similarity of HiS and LoS rats for higher-nutrition products is not due to a ceiling effect because it is apparent for fruits, and the intake of banana was well below what rats were willing and able to eat in during one test period.

A Line  $\times$  Sex  $\times$  Food Category (fruit or vegetable)  $\times$  Nutrition Level (lower or higher) ANOVA yielded main effects of line, sex, food category, and nutrition level [respectively,  $F(1, 43) = 12.50$  with  $p < .001$ ,  $8.10$  with  $p = .007$ ,  $127.50$  with  $p < .001$ , and  $50.70$  with  $p < .001$ ] and two-way interactions of line with food category and with nutrition level [respectively,  $F(1, 44) = 5.07$  and  $5.21$ ,  $ps = .03$ ]. Contrasts showed that the lower versus higher nutrition difference was significant for both fruits and vegetables, the line difference was significant for both fruits and vegetables, with a larger difference (with a smaller  $p$  value) for vegetables, and, the line difference was significant for lower-nutrition foods but not for higher-nutrition foods.

Figure 9B shows intake in the choice tests. As in the successive tests, rats ate more when vegetables were available than when fruits were available. Also, they ate more of the higher nutrition food in a category (banana or sweet potato) than the lower nutrition food in a category (pear or pea, respectively), a preference that was larger for fruits than for vegetables and among LoS rats than HiS rats. In fact, whereas LoS rats were choosy in both choice tests, HiS rats ate vegetables indiscriminately.

A Line  $\times$  Sex  $\times$  Food Category (fruit or vegetable)  $\times$  Nutrition Level Choices (lower vs. higher) ANOVA yielded main effects of food category and nutrition level choices [respectively,  $F(1, 43) = 815.49$  and  $124.37$ ,  $ps < .001$ ] and a Food Category  $\times$  Nutrition Level Choices interaction [ $F(1, 43) = 34.88$ ,  $p < .001$ ]. In addition, the main effect of line was significant [ $F(1, 43) = 28.96$ ,  $p < .001$ ], as were interactions of line with sex, food category, and nutrition level choices [respectively,  $F(1, 43) = 4.50$  with  $p = .04$ ,  $32.94$  with  $p < .001$ , and  $11.11$  with  $p = .002$ ]; the Line  $\times$  Sex  $\times$  Food Category interaction also was significant [ $F(1, 43) = 11.59$ ,  $p = .001$ ]. Contrasts confirmed the higher  $>$  lower nutrition preference for both fruits and vegetables among LoS rats but only for fruits among HiS rats. With respect to the Line  $\times$  Sex  $\times$  Food Category interaction, contrasts showed that it was ordinal: For both females and males, the line difference was significant only in the vegetable choice test.

### **Experiment 7: Macronutrient Self-Selection**

Macronutrient self-selection by HiS and LoS rats was examined as a complement to the preceding experiments on complex foods. The results of those experiments make clear that the saccharin phenotype does not predict food intake in a straightforward way. The question here was whether tests with pure macronutrients – which are seldom encountered outside of the lab – would be more straightforward. In interests of replication and external validity, each rat was tested twice with pure macronutrients, once with plant-based fat and protein and once with animal-based fat and protein. Potato starch and corn starch were used as carbohydrates. To determine the stability of intake, each set of macronutrients was tested on three consecutive days.

#### ***Materials and Procedure***

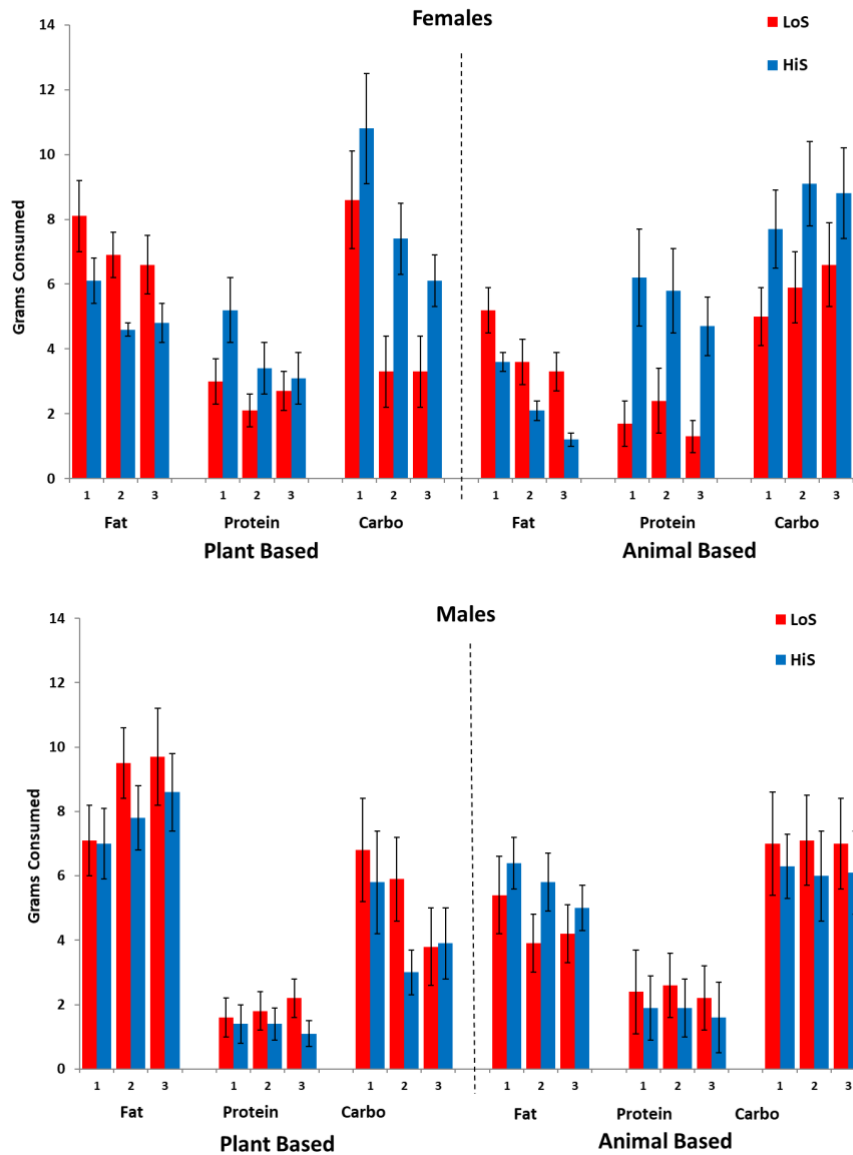
In each macronutrient test, three 2.5 oz baby food jars containing, respectively, a fat, a protein, or a carbohydrate were presented in a stainless-steel holder. In the plant-based tests, rats were given vegetable shortening (Crisco), soy protein (NOW Sports), and corn starch (Signature Select). In the animal-based tests, rats were given lard (Farmer John), casein (MP Biomedicals), and potato starch (Anthony's Organic). The position of the macronutrients was balanced across rats each day. Rats received three consecutive tests with plant sources and three consecutive tests with animal sources. The plant and animal series were separated by a day off, and test order was balanced.

#### ***Results and Discussion***

Figure 10 shows macronutrient intake for each group by plant/animal source and test day. Striking line differences are apparent among females, with LoS females consuming more fat than HiS females and HiS females consuming more carbohydrate and protein than LoS females. These patterns resemble two subpopulations of males identified by Shor-Posner et al. (1991) as, respectively, fat preferring and carbohydrate preferring – yet line differences were minimal among males. In contrast, Experiments 2A, 2C, and 3 each yielded a line difference that was observed only among males. Whether line differences are female-limited or male-limited apparently depends on food attributes.

**Figure 10**

*Intake of Pure Macronutrients Across Days 1-3 (Experiment 7)*



*Note.* Means and SEMs are shown. “Carbo” refers to carbohydrates.

Two other patterns were independent of line. The first distinguished females from males. When plant sources were available, females ate more on the first test day than they did subsequently. Overall macronutrient intake was stable across days among males. The second pattern distinguished plant from animal sources. Intake of plant protein (casein) and animal fat (lard) decreased after the first test. These changes were not unique to females, and no other change across days was significant. These results suggest that the sourcing of macronutrient matters in terms of expression of individual differences and the stability of intake across repeated exposures (Reed et al., 1992).

A Line  $\times$  Sex  $\times$  Source (Plant vs. Animal)  $\times$  Macronutrient (fat, protein, carbohydrate)  $\times$  Day (Tests 1-3) ANOVA yielded main effects of source, macronutrient, and day [respectively,  $F(1, 41) = 4.54$  with  $p = .04$ ,  $F(2, 82) = 25.06$  with GG-corrected  $p < .001$ , and  $16.30$  with  $p < .001$ ) and many interactions, including Sex  $\times$  Macronutrient, Line  $\times$  Sex  $\times$  Macronutrient, Sex  $\times$  Day, Source  $\times$  Macronutrient, Source  $\times$  Day, and Sex  $\times$  Source  $\times$  Day [respectively,  $F(2, 82) = 6.52$  with  $p = .002$ ,  $4.37$  with  $p = .02$ ,  $7.61$  with  $p < .001$ ,  $16.44$  with  $p < .001$ ,  $4.30$  with GG-corrected  $p = .02$ , and  $8.55$  with  $p < .001$ ], in addition to Source  $\times$  Macronutrient  $\times$  Day [ $F(4, 164) = 9.28$ , GG-corrected  $p < .001$ ]. The highest order interactions were interpreted with pairwise contrasts, which confirmed line differences in macronutrient self-selection only in females (LoS  $>$  HiS for fat, HiS  $>$  LoS for protein and carbohydrate) and that the following were highest on Test 1: females' macronutrient intake in the plant-based series, plant protein intake, and animal fat intake.

## General Discussion

Omnivory was on display in this study. No test food was rejected by any group of rats. Any of these test foods or, presumably, comparable foods available in other cultures would be suitable for use as part of a cafeteria diet or enrichment protocols. Some foods may be preferable on practical grounds and, to minimize variation due to differential intake, foods showing minimal group differences in this study could be considered.

Whereas laboratory rats typically are exposed to a single maintenance diet, most humans are exposed to sensory and nutritional variety. Some differences between laboratory rats and humans that have been attributed to species differences might arise, in part, from the reductionistic tradition of holding constant the dietary (and other) experiences of the former. In one illustration of how conventional maintenance diets might influence behavior, feeding mice a hamburger-supplemented diet improved memory and reduced anxiety relative to chow-fed controls, with diet-induced alterations in gut microbiota potentially mediating group differences (Li et al., 2009). Determining which prior findings hold up in laboratory rats raised with the dietary diversity characteristic of omnivores deserves scrutiny. The foods examined here can be helpful to such an agenda.

This study offers a wealth of information for researchers interested in dietary habits of Norway rats and people and the consequences thereof. However, the results are complicated and their generality may be unclear. To increase their utility, we aggregated intake of the 10 types of foods we used across the experiments and compared it intake with foods used in Shafat et al.'s (2009) cafeteria-diet study. Table 2 shows mean intake (in grams) of the ten types of food used in Experiments 1-7, averaged across varieties and arranged from highest to lowest intake (in grams). We then identified 9 foods among the 36 foods used by Shafat et al. (2009) that were most directly comparable to one of our foods (no food was comparable to pure protein). Intake of those foods is shown in Table 2. Overall intake was lower in Shafat et al.'s (2009) study than in our study for any number of reasons, such as their rats being smaller (all male, 200-250 g) and having four test foods available concurrently. Despite the many differences between studies and the small number of food types, the correlation between intake of our foods and their counterparts is nearly perfect [ $r(7) = 0.94$ ,  $p < .001$ ]. The relative intake of the foods used in the present study does not appear to be idiosyncratic to the products or custom formulations we used.

**Table 2***Mean Intake of Foods in the Present Study and Comparable Foods in Shafat et al. (2009)*

Food in Present Study	Mean Intake (in grams)	Most Comparable Food in Shafat et al. (2009)	Mean Intake (in grams)
Vegetables	125.8	Carrot	17.9
Fruits	60.8	Banana	11.0
Spam	40.0	Spam	3.4
Cream cheese	28.5	Cheese (mild cheddar)	1.1
Cookies	28.3	Cookies	0.3
Peanut butter	21.2	Peanut butter	3.6
Chocolate	10.7	Chocolate	1.8
Pure carbohydrate	6.3	Sucrose cubes	2.5
Pure fat	5.7	Lard	0.6
Pure protein	2.6	[none]	--

*Note.* Foods are arranged from highest to lowest intake in the present study (first and second column).

The present results reinforce the utility of using both successive and choice test procedures. In some cases, amounts consumed in successive tests were predictive of relative amounts consumed in the choice tests (or vice versa). Intake of fruits and vegetables is an example. However, intake of low-fat cheese, peanut butters, and Spam differed between LoS and HiS rats in the successive tests (Experiments 1A and 5) but not in the choice tests (Experiments 1B and 5). Conversely, LoS rats were choosier than HiS rats when given a choice between cream cheese versions, but the lines consumed similar amounts of all three versions in the successive tests (Experiment 2B). These findings illustrate how the food landscape sets boundary conditions on the expression of individual or group differences.

Perhaps the least surprising finding with respect to line differences is that HiS rats ate more of some high-carbohydrate foods (such as chocolate) than did LoS rats. Straightforward predictions based on sweetness, however, fell short. For instance, cookie intake did not differ between lines in Experiment 1A or in successive tests in Experiment 2C. Also, whereas the saccharin phenotype robustly distinguishes LoS and HiS rats of either sex (Dess & Chapman, 2020), some line differences were significant only among females or only among males. No prior research set an expectation that LoS rats would consume more meat than the HiS rats. Shifting from aqueous solutions and standardized diets to diverse foods revealed new, unexpected line similarities and differences.

When LoS and HiS rats differed, LoS rats were choosier about high-fat foods than were HiS rats, and HiS rats consumed high-carbohydrate foods more avidly than did LoS rats. LoS rats' choosiness and HiS rats' avidity were both expressed when foods were high in protein. These patterns can be seen in the two-dimensional array in Table 3. The ten types of food are arranged by intake (highest to lowest) and along a crude macronutrient spectrum (from pure fat to pure carbohydrate), and the ten Intake × Macronutrient intersections are populated with significant line differences. Sex-specific line differences that were inconsistent across food versions are omitted from this visualization. LoS rats' choosiness clusters on the fat side of the spectrum whereas HiS rats' avidity clusters on the carbohydrate side of the spectrum. Both dietary propensities are apparent in the protein band of the spectrum. Table 3 also shows that the lines differed significantly for every type of food.

**Table 3**

*Line Differences Arrayed by Ranked Food Intake and a Macronutrient Spectrum*

Intake Rank	Fat			Protein			Carbohydrate			
	Pure Fat	Spam	Cheese	PB	Pure Pro	Vegs	Cookies	Choc	Fruits	Pure Carb
#1 Vegetables						HiS >LoS, LoS choosier				
#2 Fruits									HiS >LoS	
#3 Spam		LoS >HiS								
#4 Cheese			HiS>LoS, LoS choosier							
#5 Cookies							HiS >LoS			
#6 Peanut butter				HiS>LoS, LoS choosier						
#7 Chocolate								HiS >LoS		
#8 Pure carb										HiS>LoS females
#9 Pure fat	LoS>HiS females									
#10 Pure protein					HiS>LoS females					

*Note.* The left column shows foods in the present study ranked by amount consumed (#1 = highest intake in grams). The top two rows show a crude macronutrient spectrum (from pure fat to pure carbohydrate). Entries in **blue** indicate a higher mean on the index among HiS rats, and entries in **red** indicate a higher mean on the index among LoS rats.

Notably, each line's propensity manifested independently of whether overall intake of the food was relatively high or low (i.e., higher to lower in Table 3). The segregation of LoS and HiS rats primarily along the macronutrient spectrum suggests that the line differences reflect responsiveness to food composition, not an underlying palatability dimension. Expression of sex-specific line differences, on the other hand, does vary from lower-intake to higher-intake foods. Female-limited line differences were expressed in tests with pure macronutrients, which were consumed in relatively small amounts. Male-limited line differences

were expressed inconsistently across food versions and test procedures (and thus are omitted from Table 3), but, when they were observed, it was in tests with foods consumed in intermediate quantities (peanut butter, cheese, cookie). This pattern of results suggests that food complexity minimizes dispositional differences among females and amplifies them among males.

Since its inception, our HiS/LoS selective breeding project has been guided by a behavioral systems approach in which ingestive behavior is integrated with the navigation of threats and resources other than food, with energy regulation as a common currency (Dess & Minor, 1996; Dess et al., 2000; Dess et al., 2007; Dess et al., 2018; also see Schneider et al., 2013). Consistent with this approach, we have observed line differences in noningestive behaviors such as responses to psychoactive drugs, acoustic startle amplitude, defensive strategies in an open field, and social interaction (Carroll et al., 2008; Dess et al., 2000; Dess et al., 2020; Dess et al., 2005; Eaton et al., 2012; Gonzales et al., 2008). The present study builds on those findings by demonstrating that selective pressure on a taste phenotype yields divergent dietary propensities that provide quick energy (carbohydrates) or energy reserves (fats). Further inquiry into the functional and mechanistic relationships between eating and other appetitively and aversively motivated behaviors is warranted. A richer food landscape in the laboratory should be part of that effort.

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