Overeating by Young Obesity-prone and Lean Rats Caused by Tastes Associated With Low Energy Foods

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Abstract

PIERCE, W. DAVID, C. DONALD HETH, JOANNA C. OWCZARCZYK, JAMES C. RUSSELL, AND SPENCER D. PROCTOR. Overeating by young obesity-prone and lean rats caused by tastes associated with low energy foods. *Obesity.* 2007;15:1969–1979.

Objective: Childhood obesity is a prominent health problem that may involve early learning about tastes and the energy content of foods. We tested the hypothesis that food tastes predictive of low energy content cause overeating in young animals.

Research Methods and Procedures: Juvenile and adolescent (4- and 8-week-old) male JCR:LA-cp lean (+/cp or +/+) and obesity-prone (cp/cp) rats were given sweet (saccharin) and salty (sodium chloride) gelatin cubes made with starch (high caloric) or no starch (low caloric) for 16 days of taste conditioning. After 10 hours of food deprivation, rats received pre-meals with flavors that had been paired or unpaired with high caloric content during taste conditioning, followed immediately by measurement of chow intake at regular meals.

Results: Our findings show that both lean (+/cp) and obesity-prone (cp/cp) juvenile rats ate more regular chow after a pre-meal with a flavor associated with low caloric value than after a similar pre-meal with a flavor predictive of high caloric content. This effect occurred with juvenile rats but not with adolescents.

Discussion: Data from our study indicate that the subversion of the relationship between taste and caloric content disrupts the normal physiological and behavioral energy balance of juvenile rats, resulting in overeating that is independent of genetic disposition for obesity.

Key words: childhood obesity, animal models, genetic susceptibility, eating behaviors, behavioral science

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Introduction

Obesity is a significant risk factor for both type 2 diabetes and cardiovascular disease and is an increasing major health problem of North America and Europe (1–5). In this regard, it is important to note that the food industry generates an extensive variety of products, some of which offer attractive tastes but have little or no nutritional value (i.e., low caloric content) (6). Indeed, it has become commonplace for people to eat "calorie-wise" foods and drinks rather than consume diets of high energy content (primarily sugar and fats). The shift to low energy-density foods and drinks is usually perceived as a positive step for weight control and is promoted today as a means to reduce the escalating levels of adult and childhood obesity in American society (7). However, a recent epidemiological review by Fowler et al. (8) of 26 years of data collected from heart patients at the University of Texas Health Sciences Center indicates that the use of low-calorie products is not always beneficial for weight control. The Texas group found that drinking low energy (non-sugar-containing) soft drinks is more strongly correlated with obesity than drinking ordinary soft drinks containing high levels of sugar.

The increased risk of obesity from low-calorie drinks may involve the subversion of the usual relationships among tastes (sweetness), caloric content, and energy intake. In this regard, an important question is whether children and adolescents inadvertently become susceptible to overeating from early experiences with food tastes, contributing to the substantial increase in childhood obesity (9,10). Given the ubiquity of the "diet craze," many children may learn that the taste of food does not predict its energy value. Children with such a dietary history may not effectively regulate their caloric intake over the course of a day: overeating at dinner, by failing to compensate for intake of palatable high-calorie snack foods during and after school (11,12). Such inability to regulate intake at meals based on assessed caloric intake might underlie the results of the University of Texas study.

To unravel the behavioral factors that contribute to overeating and obesity, Davidson and Swithers (13) investigated the effect of consistency between sweetness and caloric content of soft drinks in weanling rats. For rats in the consistent learning group, sweetness always predicted caloric content, the soft drinks contained glucose or sucrose that alternated over days of taste conditioning. In contrast, sweetness only partially predicted energy content for the inconsistent animals, the soft drinks were made with sucrose on some days and saccharin (non-caloric) on others. After taste conditioning, all rats were given a sweet nutritious pre-meal followed by access to regular food. Young rats given the inconsistent training ate substantially more of their familiar food than consistently trained animals. That is, low predictability between sweetness and caloric content resulted in overeating of familiar food by young animals.

A central question for the present study is whether taste conditioning and overeating by rats is specific to the sweetness-caloric content relationship (14). In nature, sweetness and nutritional value are highly correlated (e.g., in berries and other fruit), perhaps making weanling rats and other animals particularly susceptible to subversion of the usual predictive relationship between sweet tastes and calories. Swithers et al. (15) were unable to obtain an overeating effect in juvenile rats (≈30 days at the start of training) when food items with fatty tastes were inconsistently associated with caloric content. Adult rats given inconsistent fatty taste-calorie experience ate as much after a high calorie pre-meal as without a pre-meal while adult rats given consistent experience reduced intake after the pre-meal. Inconsistency between taste and calories resulted in weaker compensation for the energy rich pre-meal in adult animals. The researchers noted that the observed differences in response between young and adult rats in the inconsistent group probably reflected differences in experimental design with adults having fewer sensory cues to predict caloric content.

Although there is evidence that rats can discriminate tastes of free fatty acids (16), a test based on the more common taste modalities could be more sensitive. A taste that is often found in human foods is sodium chloride or "salt"; this substance is included in foods that have high nutritional value as well as in diet foods that have little or no caloric content. The present study sought to establish whether sweet and salty tastes that signaled the presence or absence of caloric content would differentially affect rats' eating of familiar food.

Another question concerns the generality of the overeating effect in lean and obesity-prone rats. The genome of the JCR:LA-cp strain of rats contains the autosomal recessive *cp* gene first isolated by Koletsky (17). Rats that are heterozygous (+/cp) or homozygous normal (+/+) are lean, while those that are homozygous for the *cp* gene (cp/cp) become obese, insulin resistant, and hypertriglyceridemic (18,19). These physiological differences could make obesity-prone (cp/cp) rats more sensitive than lean rats to tastes signaling the presence (or absence) of caloric content. Greater sensitivity, in turn, might result in more overeating by obesity-prone rats when compared with lean animals.

In the present study, lean and obesity-prone JCR:LA-cp rats were conditioned to tastes using consumable items, in an attempt to extend the overeating effect beyond flavored drinks to food stimuli. The hypothesis is that juvenile and adolescent rats overeat their regular food after a pre-meal containing a flavor previously associated with low energy content compared with a similar snack containing a flavor predictive of high caloric content.

Research Methods and Procedures

Approach

Our test of the research hypothesis uses gelatin cubes as the consumable items. A major advantage of gelatin cubes is that both tastes and caloric content can be manipulated separately. Drinks flavored with sugars such as glucose and sucrose raise experimental problems as these substances are not equal in sweetness and are absorbed via different mechanisms and at different rates. In flavored drinks, these differences entangle the conditioned stimulus (sweetness) with the unconditioned stimulus (calories), which restricts experimental analysis of the animal model.

Our procedures have an additional advantage. We were able to compare the overeating effect within the same animals, rather than between groups of rats. Comparison of paired and unpaired flavors within an animal allowed for a more direct manipulation of predictability between the conditioned stimulus-taste and the unconditioned stimulus-calories than with the partial pairing or inconsistency procedure of previous studies. With regard to the possible confounding of taste-calorie predictability, note that rat pups given inconsistent pairings of sweetness and calories also experienced greater caloric variability than consistently trained animals. Caloric variability may have induced metabolic or other physiological changes that added to, or combined with, the inconsistent pairing procedure to increase food intake of animals in this group.

Animals

The Juvenile Study concerned overeating by young lean rats and was designed to test the overeating hypothesis using food tastes rather than sweetened drinks. For this experiment, we used 16 lean (+/cp or ++) male JCR:LA-cp rats, ~21 days of age on arrival from our established breeding colony at the University of Alberta. The Obese Study extended the overeating effect to young obese animals, using 8 obesity-prone (cp/cp) and 8 lean juvenile rats; the Adolescent Study investigated overeating with adolescent rats to examine the generality of the overeating effect by age; we used 16 adolescent lean male rats (38 to 40 days old on arrival) in each replication. Animals were housed individually in a temperature- (22 ± 2 °C) and humidity-controlled (55%) environment and maintained on a standard 12-hour reversed light/dark cycle (lights off at 7:30 AM when feeding tests occurred). Access to food and water was as outlined in the procedure. The care and use of animals were in accordance with the Guidelines of the Canadian Council of Animal Care and subject to prior approval by University of Alberta Animal Welfare and Policy Committee.

Housing

The rats were housed in transparent polycarbonate cages (40.6 × 15.2 × 22.9 cm) with wood-chip bedding and metal tops that allowed for food provisioning and water bottles. Feeding cages were exactly the same without bedding.

Foods

Three types of food were used in these experiments: standard laboratory chow (LabDiet 5010-Autoclavable Rodent Diet; PMI Nutrition International, Inc., Brentwood, MO), gelatin cubes made with grape-flavored powder (Kool-Aid; Kraft Canada, Inc., Don Mills, ON, Canada) for the Juvenile Study or without grape flavor (the Obese and Adolescent studies), and commercial rice cakes (Quaker, original whole grain brown rice, 3.9 kcal/g; Pepsi-QTG Canada, Mississauga, ON). Details of the preparation of foods and caloric content are given in Appendix A (available online at the *Obesity* website, www.obesityresearch.org).

Acclimatization, Conditioning, and Tests

Acclimatization. For the 5 days of acclimatization (7 days for the Juvenile Study), the rats were habituated to the experimental room and handling; the animals were fed in home cages with standard laboratory chow (20 g/d) and water freely available. For 3 days (or 5 days of the Juvenile Study), rats were exposed to gelatin cubes without added tastes or calories for 4 hours in feeding cages and then returned to their home cages for 18 hours of access to the chow and water. On the last day of acclimatization, rats were pair matched in terms of body weights and randomly assigned to training groups.

Taste Conditioning and Deprivation. The animals assigned to the Sweet Paired group received 8 days of cubes made with saccharin and starch and 8 days with salt but no starch. For the Sweet Paired group, sweetness was paired with calories and salty taste was not (unpaired). The Salt Paired group received 8 days of cubes made with salt and starch, and 8 days of cubes with saccharin and no starch. For the Salt Paired group, salty taste was paired with calories and sweetness was not (unpaired).

During the conditioning period (16 days), cubes were given for 4 hours in feeding cages; subsequently, animals were returned to home cages with chow and water. For all rats, two sweet (A) and two salty (B) cubes were presented in blocks of 4 days, according to the sequence ABBAAB-BABAABBAAB (20). After the conditioning phase, all animals were given 38 hours of access to chow (30 g) and water in home cages to stabilize body weights before overeating tests. Then, chow and water were withdrawn for 10 hours to establish motivation to eat the pre-meal snacks and chow on test days.

Tests for Overeating. On the next day at 8:00 AM, all rats were given the first of two food tests. For the Juvenile Study, the initial test began with a 1-hour pre-meal of salty rice cake in feeding cages. The pre-meal exposed animals to a nutritious and filling snack with a taste that had been paired or unpaired with calories during the conditioning phase, depending on the experimental condition. Animals then were transferred to home cages with 30 g of chow and water to test for overeating of familiar food. Measures of chow and water intakes were taken for 3 hours after the return of regular food and water. After another 38 hours of weight stabilization and 10 hours of food deprivation, a second overeating test was conducted with saccharin (sweet) added to the rice cakes for taste. For the Obese and Juvenile studies, one half the rats received salty pre-meals on the first test and sweet on the second. This was reversed for the other rats.

Body Weight and Food Intake Measures

An electronic scale (Model PJ3600; Mettler-Toledo, Inc., Columbus, OH) was used to measure food, water, and body weight to the nearest gram. For the entire experiment, these measures were recorded every 24 hours, beginning at 10:00 AM; intake of cubes in feeding cages was measured after 4 hours. For overeating test days, the pre-meal intake of rice cakes was measured after 1 hour. Intake of chow (30 g) after the pre-meals was measured after 1, 2, and 3 hours.

Juvenile Study: Overeating by Young Lean Rats. Two groups of JCR:LA-cp lean juvenile rats differed in taste conditioning. Over days, one group received gelatin cubes that contained saccharin and starch or salty cubes without starch (Sweet Paired, $N = 8$). The other group received gelatin cubes made with sodium chloride and starch or saccharin cubes without starch (Salt Paired, $N = 8$). Next, all rats received pre-meals of rice cakes dipped in saccharin and water, followed by a test for consumption of standard chow. After a period of recovery, all rats were given pre-meals of rice cakes dipped in salty water, followed by a

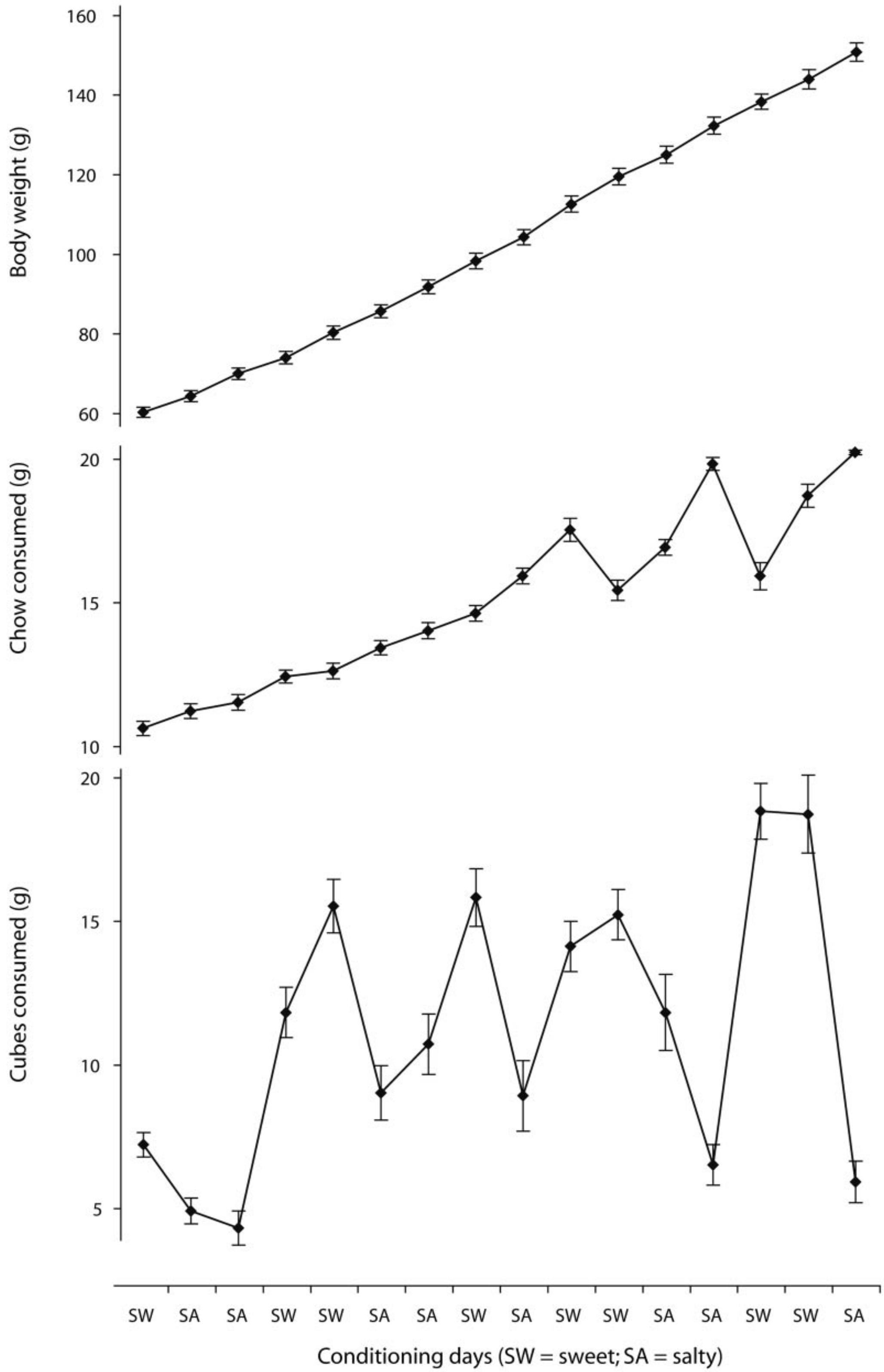

Figure 1: Body weight, chow intake, and consumption of cubes (g) over 16 days of conditioning with sweet (SW) and salty (SA) flavors.

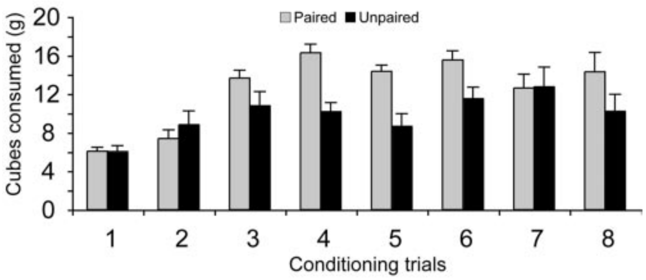

Figure 2: Intake of flavored cubes (g) on paired and unpaired conditioning trials.

second test for consumption of regular chow. All rats were randomized to treatment conditions.

Obese Study: Overeating by Young Obese Rats. Four groups of rats were compared: JCR:LA-cp lean (+/cp or ++) and Sweet Paired, JCR:LA-cp lean and Salt Paired, JCR:LA-cp obesity-prone (cp/cp) and Sweet Paired, and JCR:LA-cp obesity-prone and Salt Paired. The procedures for conditioning and tests were as in the Juvenile Study, except that on a random basis half the rats received sweet pre-meals on the first test and salt pre-meals on the second; the others received the reverse order of flavors.

Adolescent Study: Overeating by Adolescent Rats. The groups and procedures were as in the first study but rats were 38 to 40 days old on arrival, rather than juveniles. For acclimatization, animals received 2 or 4 days of exposure to clear gelatin cubes. Rats in the Adolescent(a) Study received larger portions of rice cakes and more of the sweetener in the cubes and cakes, or, in the Adolescent(b) Study, received the same amounts as in the first study (Appendix A, available online at the *Obesity* website, www.obesity research.org). On a random basis, half of the rats received sweet pre-meals on the first test and salt pre-meals on the second; the others received the reverse order of flavors.

Statistical Analyses

Body weight, chow intake, and consumption of cubes for acclimatization were analyzed with ANOVA. The Juvenile and Adolescent studies used a one-factor (days) repeated-measures design; the Obese Study was a mixed design using 2 (lean vs. obesity-prone) by 5 (days) ANOVAs. For the taste conditioning phases, we used 2 (paired vs. unpaired) by 16 (days) repeated-measures ANOVAs to analyze body weight, chow intake, and consumption of cubes; we also conducted 2 (paired vs. unpaired) by 8 (trials) repeated-measures ANOVAs on intake of cubes. Analyses of chow intakes preceding food deprivation, body weights after the 10 hours of deprivation and intake of rice cakes for the pre-meals were conducted with one-factor (paired vs. unpaired) repeated-measures ANOVAs. Chow intake 3 hours after the pre-meals was analyzed with 2 (salt vs. sweet) by 2 (paired vs. unpaired) ANOVAs for the Juvenile and Adolescent studies; we used a 2 (lean vs. obesity-prone) × 2 (paired vs. unpaired) design for the chow test of the Obese Study. Significance levels were set at 0.05 (two-tailed) for all tests, except for planned contrasts on chow intake after the pre-meals for the Juvenile and Obese studies ($\alpha = 0.05$, one-tailed), as an increase was predicted for young rats on the basis of previous research (13,14). Descriptive statistics are given as mean ± standard error.

Results

Juvenile Study

Acclimatization. During the acclimatization period, food intake [$F(6,90) = 196.67$, $p < 0.001$] and body weights [$F(6,90) = 561.13$, $p < 0.001$] increased over 7 days. Mean food intake on the last day of acclimatization was 9.7 ± 0.24 g, and mean body weight was 56.1 ± 1.28 g. Intake of the gelatin cubes showed a step-like increase over days [$F(5,75) = 6.71$, $p < 0.001$]: juvenile rats ate ~1 g of the cubes for the first 3 days of exposure and ~2 g subsequently.

Taste Conditioning and Deprivation. Figure 1 depicts body weight, chow intake, and consumption of gelatin cubes (g) over the 16 days (trials) of conditioning by order of flavor. Juvenile rats gained body weight (top) and increased chow (middle) over trials. Notably, the intake of flavored cubes (bottom) varied with the taste, rats preferring sweetened over salty cubes. During the conditioning phase, chow intake increased over days regardless of pairing [$F(7105) = 409.41$, $p < 0.001$], as did body weight [$F(7,105) = 450.73$, $p < 0.001$]. Figure 2 depicts cube consumption for the 8 paired and 8 unpaired conditioning trials. Intake of the flavored cubes showed a significant interaction of taste conditioning (paired vs. unpaired) by trials [$F(7,105) = 4.68$, $p < 0.001$]. For the days of food

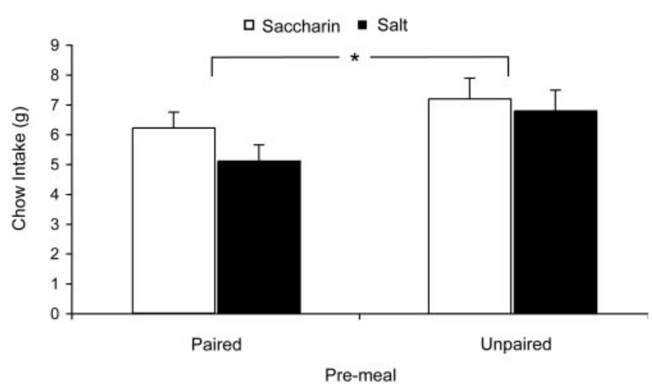

Figure 3: Consumption of chow on the tests as a function of taste conditioning (paired vs. unpaired) and flavor (saccharin vs. salt) of the pre-meal. * $p < 0.01$ (one-tailed) for the paired vs. unpaired effect.

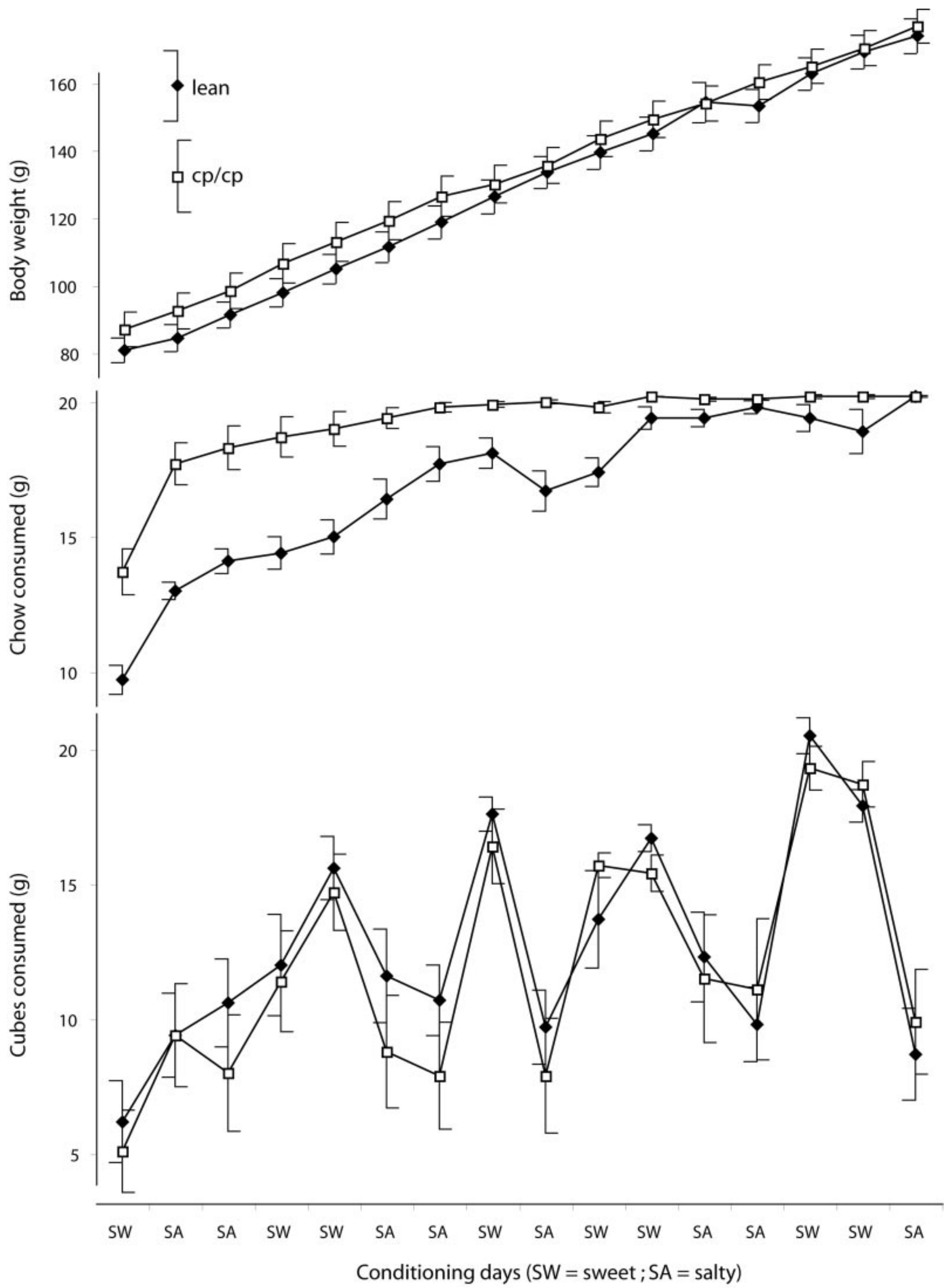

Figure 4: Body weight, chow intake, and consumption of cubes (g) over 16 days of conditioning with sweet (SW) and salty (SA) flavors by lean and obesity-prone (cp/cp) rats.

deprivation (10 hours), there was a significant effect of taste conditioning on chow intakes for the period (7:30 AM to 10:00 AM) preceding food removal [$F(1,15) = 8.13$, $p < 0.02$]. Juvenile rats ate significantly more on the day that deprivation began when tastes had been paired with caloric content, rather than unpaired (12.6 ± 0.50 g and 11.2 ± 0.42 g, respectively). The ANOVA on body weights after 10-hour periods of deprivation, however, showed no significant effect of taste conditioning [paired vs. unpaired; $F(1,15) = 0.02$, not significant]. The juvenile rats were similar in body weights (165.1 ± 2.23 g) before the paired and unpaired tests for overeating.

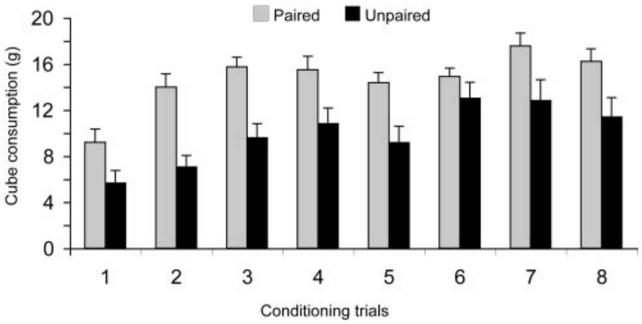

Figure 5: Intake of flavored cubes (g) on paired and unpaired conditioning trials.

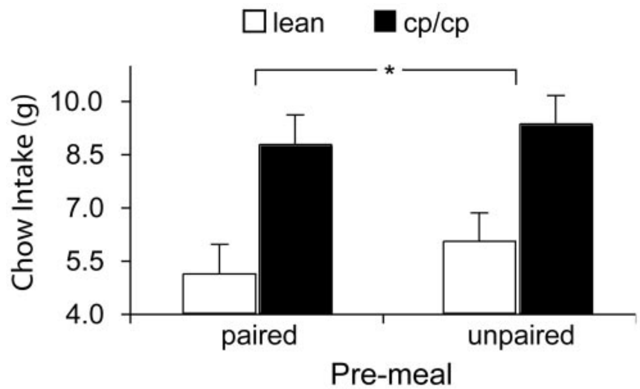

Figure 6: For lean and obesity-prone (cp/cp) rats, consumption of chow on the tests as a function of taste conditioning (paired vs. unpaired) and flavor (saccharin vs. salt) of the pre-meal. * $p < 0.05$ (one-tailed) for the paired vs. unpaired effect.

Tests for Overeating. There was no significant difference in consumption of flavored rice cakes (tasty snacks) between paired and unpaired pre-meals. Mean consumption was 5.9 ± 0.15 g. Figure 3 depicts chow intake (g) in home cages for the 3 hours after the pre-meal. As expected, chow intake was greater when the flavor of the pre-meal had predicted fewer calories (unpaired, 7.0 ± 0.49 g) than when it had predicted more [paired, 5.6 ± 0.38 g, t (15) = 2.70, $p < 0.01$, one-tailed]. These results indicate an overeating effect for juvenile rats.

Obese Study

Acclimatization. During the acclimatization period, food intake [$F(4,52) = 112.66$, $p < 0.001$] and body weights [$F(4,52) = 594.17$, $p < 0.001$] increased over 5 days for both genotypes. The days-by-genome (lean vs. obesity-prone) interaction effects for food intake [$F(4,52) = 3.85$, $p < 0.01$] and body weights [$F(4,52) = 9.72$, $p < 0.001$] were significant, indicating that obesity-prone rats increase their food consumption and body weights over days more than lean animals. Mean chow intake (g) on the last day of acclimatization was 12.8 ± 0.48 and 17.6 ± 0.82 for lean and obesity-prone animals, respectively. Body weights (g) were 74.5 ± 3.25 and 84.1 ± 3.80 for these same animals. Intake (g) of the gelatin cubes (Days 2 to 5) did not differ significantly by days, genome, or days-by-genome; rats ate 1.7 ± 0.36 g of the cubes, on average.

Taste Conditioning and Deprivation. Figure 4 depicts body weight, chow intake, and consumption of flavored cubes over the 16 trials for lean and obesity-prone rats. The figure also shows the order of presentation of sweet and salty cubes. Body weights (top) of obesity-prone rats were initially greater than lean animals but the differences diminish over days, due to the 20-g limit for daily chow. Obesity-prone rats ate most of the 20 g of chow (middle) by Day 7 of conditioning; lean rats took up to Day 13 to eat this much chow. Lean and obesity-prone animals consumed more of the sweet cubes than the salty ones (bottom); lean and obesity-prone rats ate similar amounts of sweet cubes, but obesity-prone animals ate less of the salty cubes than lean rats. Generally, obesity-prone rats preferred sweet over salty tastes more than lean animals.

The intake of the flavored cubes (Figure 5) showed a significant main effect of taste conditioning [paired vs. unpaired; $F(1,14) = 11.16$, $p < 0.005$]. Rats ate more of the flavored cubes that were paired with calories (14.7 ± 0.70 g) than the cubes with low nutritional content (9.9 ± 1.1 g). There was a significant effect of conditioning trials as intake of flavored cubes increased at first and then leveled off [$F(7,98) = 25.85$, $p < 0.001$].

We conducted ANOVAs for the effects of taste conditioning (paired vs. unpaired) and genome (lean vs. obesity-prone) on the rats' chow intakes preceding food deprivation and body weights after the 10-hour deprivation periods (before consumption of the pre-meals). There was a significant effect of genome on chow intake for deprivation days preceding the tests [lean = 15.2 ± 0.62 g; obesity-prone = 19.0 ± 0.62 g; $F(1,14) = 19.13$, $p < 0.001$]. In terms of body weight after 10 hours of food deprivation, obesity-prone rats weighed 199.8 ± 6.4 g and 199.3 ± 6.4 for the paired and unpaired tests, respectively; lean rats weighed 189.0 ± 6.4 g and 187.5 ± 4.9 for these tests. The main effect for genome (lean vs. obesity-prone) and the taste conditioning (paired vs. unpaired)-by-genome interaction were not statistically significant, indicating that body weights were similar before the pre-meals and tests for overeating.

Tests for Overeating. Analysis of lean and obesity-prone rats' pre-meal intakes (Tests 1 and 2) indicated there was no significant difference in consumption of flavored rice cakes between paired and unpaired tests (2.5 ± 0.11 g, 2.6 ± 0.10 g, respectively). On average, animals consumed 2.5 ± 0.08 g of the rice cake wedges. There were no significant differences in rice cake intake by lean (2.6 ± 0.12) or obesity-prone (2.6 ± 0.11) animals.

Table 1. Food intake and body weight by experimental phase for the Adolescent Study

Phase	Measure	Condition	Experiment: Adolescent(a) (g) (mean ± SE)	Adolescent(b) (g) (mean ± SE)
Acclimatization	Weight		155.1 ± 2.5	134.9 ± 3.6
	Chow		19.9 ± 0.4	17.8 ± 0.4
	Cubes		1.4 ± 0.1	1.4 ± 0.2
Taste Conditioning	Weight		235.2 ± 3.3*	219.7 ± 3.9
	Chow	Paired	18.8 ± 0.3	17.7 ± 0.3
		Unpaired	20.1 ± 0.2	18.7 ± 0.3
	Cubes	SW-paired	15.7 ± 2.2	16.0 ± 1.4
		SW-unpaired	11.0 ± 1.1	14.7 ± 1.2
		SA-paired	12.6 ± 0.9	16.0 ± 1.5
		SA-unpaired	19.2 ± 0.7	14.7 ± 1.3
Deprivation	Weight	Paired	242.2 ± 3.6	232.2 ± 4.1
		Unpaired	242.1 ± 3.4	232.1 ± 5.1
	Chow	Paired	14.0 ± 0.8	17.1 ± 0.6
		Unpaired	14.4 ± 0.6	16.8 ± 0.6
Overeating tests	Rice cake	Paired	4.6 ± 0.6	2.9 ± 0.1
		Unpaired	4.5 ± 0.5	2.9 ± 0.2
	Chow	Paired	5.5 ± 0.4	6.7 ± 0.4
		Unpaired	5.6 ± 0.5	6.7 ± 0.3

SE, standard error; SW, sweet; SA, salty.
* All means for taste conditioning are for the last training trials. Compared with the overall mean, the mean on the last trial provides a more accurate description of the rat's weight, chow intake, and cube consumption prior to the next phases of the experiment.

A planned contrast for chow intakes 3 hours after the pre-meals revealed that chow intake was greater on unpaired than paired tests [7.7 ± 0.69 g, 6.9 ± 0.74 g, respectively, t (15) = 2.06, $p < 0.05$, one-tailed], supporting the hypothesized overeating effect. Figure 6 depicts the results of the ANOVA for regular chow intake with pre-meal (paired vs. unpaired) and genome (lean vs. obesity-prone) as factors. The results showed a significant difference between lean (5.6 ± 0.77) and obesity-prone (9.0 ± 0.77) rats for consumption of regular chow after the pre-meal [$F(1,14) = 10.17$, $p < 0.01$]. The main effect of pre-meal (paired vs. unpaired) approached statistical significance [$F(1,14) = 4.02$, $p = 0.065$], supporting the planned contrast analysis. Both obese and lean JCR:LA-cp rats showed overeating after pre-meals with tastes predictive of low energy content.

Adolescent Study

Table 1 shows the summary statistics (mean ± standard error) by phases of the experiments. We also provide a textual description of results with *p* values for the repeated measures analyses; statements of results pertain to both Adolescent(a) and Adolescent(b) experiments except where differences are noted.

Acclimatization. For the acclimatization period, the adolescent rats' body weights increased over days, *p* values < 0.001. Chow intake increased and leveled off across days (*p* values < 0.001) or dropped off slightly for Adolescent(a). Intake of the unflavored cubes decreased over the days of exposure [$p < 0.001$ for Adolescent(a)] and did not significantly change over days of exposure for Adolescent(b). Table 1 lists the mean body weights, chow intakes, and consumption of unflavored gelatin cubes for the acclimatization phase.

Taste Conditioning and Deprivation. The adolescent rats gained body weight over the 16 days of taste conditioning ($p < 0.001$). Chow intake fluctuated over trials with more consumption of chow on unpaired than paired days of conditioning (*p* values < 0.001). Table 1 also indicates that rats' chow intakes on the last conditioning trials were greater after unpaired cubes than after paired. The intake of flavored cubes varied over trials by conditioning group ($p < 0.01$). For sweet-paired rats, consumption of paired cubes across trials was consistently greater than consumption of unpaired cubes; this was reversed for rats in the salt-paired group of Adolescent(a) that ate more of the saccharin cubes without starch (unpaired) over trials; salt-paired rats of Adolescent(b) (less saccharin in cubes) also consumed more unpaired (17.0 ± 0.6) than paired (14.4 ± 1.0) cubes for the last 3 trials of conditioning, $p = 0.02$. Table 1 shows that, on the last trials, sweet-paired rats (SW) of Adolescent(a) ate more of the paired cubes than the unpaired, while salt-paired rats (SA) did the reverse. Rats of Adolescent(b) ate more of the paired than unpaired cubes on the last trials, regardless of conditioning groups. The results for consumption of gelatin cubes suggest that the sweet taste influenced consumption of cubes as much as the taste-calorie associations in these adolescent rats.

There were no significant effects of taste conditioning on chow intakes for deprivation days. The ANOVA on body weights after 10-hour periods of deprivation and before the overeating tests showed no significant effect of taste conditioning (paired vs. unpaired). The adolescent rats were similar in body weight before the paired and unpaired tests for overeating.

Tests for Overeating. There were no significant differences in consumption between paired and unpaired flavored rice cakes given as a pre-meal. As indicated in Table 1, chow intake 3 hours after the pre-meal did not significantly differ when the flavor of the pre-meal predicted fewer calories (unpaired) than when it predicted more (paired). The results for adolescent rats failed to support the overeating hypothesis.

Discussion

A major question of the present study is whether taste conditioning and overeating by rats is specific to the sweetness-calories relationship. We found (Juvenile and Obese studies) that young JCR:LA-cp rats overeat their regular food after energy rich pre-meals with tastes that have signaled the absence of calories, regardless of whether sweet or salty flavors were used. One interpretation is that the subversion of the relationship between taste (conditioned stimulus) and calories (unconditioned stimulus) disrupts the developing physiological and behavioral energy balance of these young animals.

Our findings with food items support the research of Davidson and Swithers (13) who used sweetened solutions inconsistently containing caloric or no caloric content to induce overeating in juvenile rats. In the present study, however, the tastes were associated consistently with high or low calories, showing more directly that taste-calorie predictiveness is the basis of the overeating effect and not other factors that vary with inconsistency. We also were able to obtain the overeating effect within subjects: Animals demonstrated poor energy regulation (high intake of regular chow) when nutritious "before dinner snacks" were prepared with tastes indicative of low caloric content; these same animals showed good energy regulation when these snacks predicted the presence of caloric content. Thus, environmental contingencies based on Pavlovian conditioning could be central to the energy regulation of young animals at the individual level (13,21).

Swithers et al. (15) were unable to obtain an overeating effect in their juvenile rats when food items with fatty tastes were inconsistently followed by calories. In contrast, we were able to obtain overeating in juvenile animals with sweet and salty tastes using a procedure where tastes always predicted absence of calories (100%). One possibility is that the degree of predictiveness between taste cues and caloric content is a determinant of overeating in young rats. On the other hand, the ability of young rats to predict from sensory cues (fatty tastes) to caloric content may not be as well developed as that between basic tastes (sweet and salt) and energy value.

Adult rats in the fatty taste study (15) showed weak compensation of food intake (poor regulation of eating) using a procedure that inconsistently paired fatty tastes with food energy. We could not induce overeating, however, in adolescent rats when tastes always predicted an absence of caloric content (100%). In Adolescent(a), the rats received larger portions of rice cake snacks and more of the sweetener before the tests for chow consumption than in Adolescent(b), but neither experiment resulted in an overeating effect.

There are a number of parametric and design differences between the current and prior experiments that make comparisons difficult and perhaps premature. Evidence from our study suggests that sweetness alone regulates the intake of adolescent rats rather than the taste-caloric content relationship. One hypothesis is that tastes and sensory cues by themselves as well as the taste-caloric content relationship come to regulate the food intake as animals mature (22). These multiple and developing sources of control of food intake might account for differences over studies. Procedures that enhance the predictive relationship between tastes and food energy may be necessary to show strong and consistent overeating effects across juvenile, adolescent, and adult rats.

Another question addressed by our study concerns the generality of the overeating effect in lean and obesity-prone juvenile rats. We wondered if physiological differences could make obesity-prone (cp/cp) rats more sensitive than lean rats to tastes signaling the presence (or absence) of calories. Greater sensitivity, in turn, might result in more overeating by obesity-prone rats when compared with lean animals. Indeed, our results did not detect any significant differences between lean and obesity-prone rats on the overeating tests, although obesity-prone animals clearly ate more than lean ones on the tests. Our procedure required that the body weights of lean and c/cp animals were similar after the conditioning phase, ensuring that any differences in the test phases were due to the training differences rather than weight. One possibility, however, is that early conditioning and body weight combine to affect overeating. Once young cp/cp rats have gained substantial weight, they could become more prone to overeating induced by taste-calorie conditioning. Such a finding would have implications for childhood obesity in human infants if similar processes are activated.

Although we did not detect a difference in overeating between juvenile cp/cp and lean rats, obesity-prone rats do show the overeating effect. It is likely that overeating in obesity-prone animals is actually of more concern from a health perspective. Obesity-prone animals are predisposed to eat and gain body weight; their intakes of chow are significantly greater than lean rats of similar ages. Thus, compared with lean rats, the consistent ingestion of a small number of extra calories, induced by subverting the taste-calorie relationship, could be more devastating to weight regulation (23) and health in the obesity-prone animals.

Our findings are particularly relevant to the current rise in childhood obesity (9,10). As a result of early experience, children learn about tastes and other gustatory cues that signal the caloric contents of foods and beverages (21). The evidence of the present study and related research (13–15) suggests that young animals, including humans, can also learn about sensory cues that are predictive of the absence of calories, leading to overeating of meals when these cues are combined with foods ingested throughout the day.

Children from "diet conscious" families or families with concerns about body weight would be exposed to a supply of "calorie-wise" food items and drinks, along with energy dense foods, perhaps making these children more susceptible to learning that food flavors do not predict caloric content (20). These youngsters might not be adept at energy balance and have increased risk for overeating (24) and obesity. In a recent study, elementary school-age children were asked to recall what they had eaten over 24-hour periods; analysis revealed that diet soda consumption increased over a 2-year period and was associated with the children being overweight or gaining weight (11). This finding in children supports the longitudinal analysis of Fowler (8) of adult heart patients, with both studies implicating diet soda consumption with weight gain. Further research is necessary to discover if the correlation between diet soda and weight is actually due to overeating induced by the conditioning processes described in this paper.

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References

1. **Byrne CD.** The metabolic syndrome and type 2 diabetes. *Endocrinology.* 2005;30:139–59.
2. **Caban AJ, Lee DJ, Fleming LE, Gomez-Marin O, LeBlanc W, Pitman T.** Obesity in US workers: the National Health Interview Survey, 1986 to 2002. *Am J Public Health.* 2005; 95:1614–22.
3. **Fry J, Finley W.** The prevalence and costs of obesity in the EU. *Proc Nutr Soc.* 2005;64:359–62.
4. **Green V.** The domino effect: obesity, type 2 diabetes and cardiovascular disease. *Br J Community Nurs.* 2005;10:358–61.
5. **Morrill AC, Chinn CD.** The obesity epidemic in the United States. *J Public Health Policy.* 2004;25:353–66.
6. **Short D.** When science met the consumer: the role of industry. *Am J Clin Nutr.* 2005;82(Suppl 1):256–8.
7. **Rolls BJ, Drewnowski A, Ledikwe JH.** Changing the energy density of the diet as a strategy for weight management. *J Am Diet Assoc.* 2005;105(Suppl 1):98–103.
8. **Fowler SP, Williams K, Hunt KJ, Resendez RG, Hazuda HP, Stern MP.** Diet soft drink consumption is associated with increased incidence of overweight and obesity in the San Antonio Heart Study. *Diabetes.* 2005;54(Suppl 1):A258.
9. **Dehghan M, Akhtar-Danesh N, Merchant AT.** Childhood obesity, prevalence and prevention. *Nutr J.* 2005;4:24.
10. **Ells LJ, Campbell K, Lidstone J, Kelly S, Lang R, Summerbell C.** Prevention of childhood obesity. *Best Pract Res Clin Endocrinol Metab.* 2005;19:441–54.
11. **Blum JW, Jacobsen DJ, Donnelly JE.** Beverage consumption patterns in elementary school aged children across a two-year period. *J Am Coll Nutr.* 2005;24:93–8.
12. **Centers for Disease Control and Prevention.** Competitive foods and beverages available for purchase in secondary schools: selected sites, United States, 2004. *MMWR Morb Mortal Wkly Rep.* 2005;54:917–21.
13. **Davidson TL, Swithers SE.** A Pavlovian approach to the problem of obesity. *Int J Obes Relat Metab Disord.* 2004;28: 933–5.
14. **Tordoff MG, Friedman MI.** Drinking saccharin increases food intake and preference: IV. Cephalic phase and metabolic factor. *Appetite.* 1989;12:37–56.
15. **Swithers SE, Doerflinger A, Davidson TL.** Consistent relationships between sensory properties of savory snack foods an calories influence food intake in rats. *Int J Obes Relat Metab Disord.* 2006;30:1685–2.
16. **Gilbertson TA.** Gustatory mechanisms for the detection of fat. *Curr Opin Neurobiol.* 1998;8:447–52.
17. **Koletsky S.** Obese spontaneously hypertensive rats: a model for the study of atherosclerosis. *Exp Mol Pathol.* 1973;19:53–60.
18. **Russell JC, Graham S, Hameed M.** Abnormal insulin and glucose metabolism in the JCR:LA-corpulent rat. *Metabolism.* 1994;43:538–43.
19. **Russell JC, Koeslag DG, Amy RM, et al.** Plasma lipid

secretion and clearance in the hyperlipidemic JCR:LA-corpulent rat. *Arteriosclerosis.* 1989;9:869–76.
20. **Underwood BJ.** *Experimental Psychology.* Norwalk: CT: Appleton-Century Crofts; 1966, pp. 38–9.
21. **Swithers SE, Davidson TL.** Obesity: outwitting the wisdom of the body? *Curr Neurol Neurosci Rep.* 2005;5:159–62.
22. **Myers KP, Hall WG.** Conditioned changes in appetitive and consummatory responses to flavours paired with oral or nutrient reinforcement among adult rats. *Physiol Beh.* 2000;68:603–10.
23. **Woods SC, Ramsay DSS.** Pavlovian influences over food and drug intake. *Behav Brain Res.* 2000;110:175–82.
24. **Wardle J, Guthrie C, Sanderson S, Birch L, Plomin R.** Food and activity preferences in children of lean and obese parents. *Int J Obes Relat Metab Disord.* 2005;25:971–7.