

## ORIGINAL ARTICLE

## Distinct phenotypes of obesity-prone AKR/J, DBA/2J and C57BL/6J mice compared to control strains

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**Objective:** To characterize and compare three obesity-prone inbred strains, AKR/J, DBA/2J and C57BL/6J, to three control strains, C3H/HeJ, BALB/cByJ and C57L/J, selected based on their normal eating patterns and moderate weight gain on high-calorie diets.

**Methods and procedures:** These six strains were examined at 5 weeks of age while still of normal body weight, and they were maintained for 1 day or 3 weeks on different feeding paradigms with macronutrient diets. Measurements were taken of macronutrient intake, body weight and body fat accrual, circulating hormones and metabolites, and the hypothalamic peptide, galanin.

**Results:** The three control strains each selected a balanced diet with 50% carbohydrate and 15–25% fat when given a choice of macronutrients, and they had similar, normal range of scores for the measures of body weight, adiposity, the hormones, insulin and leptin, and the metabolites, glucose and triglycerides. When compared to this control baseline, the obesity-prone strains with similar total caloric intake to controls selected a diet with significantly more fat (30–40%) and less carbohydrate (<40%). They also had greater adiposity, with the largest differences detected for the AKR/J and DBA/2J strains. These two obesity-prone strains compared to control strains had elevated levels of insulin and leptin. They also had higher triglyceride levels and increased expression and levels of galanin in the hypothalamic paraventricular nucleus. A very different pattern was detected in the obesity-prone C57BL/6J strain, which exhibited a stronger preference for protein as well as fat, normal levels of insulin, leptin and triglycerides, hyperglycemia relative to all other strains, and a small increase in galanin.

**Conclusion:** These comparisons to control strains revealed a distinct phenotype in the two obesity-prone strains, AKR/J and DBA/2J, which is very similar to that described in obesity-prone, outbred rats. They also identified a clearly different phenotype in the obesity-prone C57BL/6J strain.

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## Introduction

A primary goal of research in the area of obesity is to understand factors that are causally related to this disorder and possibly involved in the early stages of its development. To accomplish this goal, one must examine obesity-prone animals prior to their gaining significant body weight. Studies of inbred mouse strains are helpful in this regard. Owing to their genetic specificity, strains with high vs low risk for obesity can be investigated at an early age, before major disturbances in body weight regulation develop. There are several studies that have characterized inbred mouse strains with a differential propensity toward obesity.<sup>1–7</sup> These

investigations have found certain strains, including AKR/J, DBA/2J and C57BL/6J, to be susceptible to the obesity-promoting effects of high-fat/high-calorie diets and other strains, most notably SWR/J and A/J, to be resistant to obesity and relatively unresponsive to these diets. A third set of mice had no distinguishable characteristics, falling somewhere in between the prone and resistant strains.

Studies to date have generally compared only two strains, an obesity-prone to an obesity-resistant, and they have examined these strains at an age when they are already different in body weight. The obesity-prone AKR/J strain, most commonly compared to the SWR/J strain, is found to exhibit a stronger preference for dietary fat, heavier body weight and adipose depots, higher insulin levels on a high-fat diet, and greater insulin-stimulated glucose transport in adipose tissue.<sup>2,8–10</sup> Also, the obesity-prone C57BL/6J mice, generally compared to the obesity-resistant A/J strain, show a similar endocrine phenotype and additionally exhibit an

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increased propensity towards diabetes,<sup>11–13</sup> along with decreased  $\beta$ -adrenergic-induced lipolysis on a high-fat diet<sup>14</sup> and reduced uncoupling protein-1 and -2 expression in brown adipose tissue.<sup>5</sup> There is little information available, however, on the DBA/2J strain. Direct comparisons between the obesity-prone strains show that the C57BL/6J mice differ in some respects from the AKR/J mice, in showing lower levels of insulin and leptin and greater hyperglycemia on a high-fat diet.<sup>15</sup>

While suggestive of mechanisms that may promote obesity, these direct comparisons between an obesity-prone and an obesity-resistant strain, with no intermediate control baseline, leave one questioning whether the differences actually reflect disturbances specifically in the prone strain rather than responses characteristic of the resistant strain. The mechanisms underlying a propensity vs resistance to obesity may not fall along a continuum. Also, since the two strains when compared as adults are already considerably different in body weight, it is not clear whether the differences observed are actually a consequence rather than a cause of the differential weight gain. Since the inbred mice constitute an important tool for investigating mechanisms of obesity, we made specific efforts in the present investigation to establish a set of 'control' mice that may be used as a baseline for direct comparisons with the obesity-prone mice. The strains selected for this control baseline were among a set of mice that were found to have specific behavioral patterns that lie within an intermediate or 'normal' range, in between the prone and resistant mice. These strains, C3H/HeJ, BALB/cByJ and C57L/J, show moderate weight gain and total intake on a high-fat/high-calorie diet,<sup>1,3</sup> and as demonstrated so far for BALB/cByJ and C57L/J, they spontaneously select a balanced macronutrient diet with a stronger preference for carbohydrate over fat.<sup>3,7</sup> To our knowledge, there are only two studies that have directly compared the obesity-prone mice to these control strains. There is one report from this lab<sup>7</sup> showing the AKR/J compared to C57L/J control mice to have a stronger preference for fat (33% vs 15%) and weaker preference for carbohydrate (25 vs 62%), along with heavier fat pad weights and elevated leptin and triglycerides on a high-fat diet. Another study<sup>16</sup> showed the AKR/J and C57BL/6J mice compared to the C3H/HeJ control strain to have higher glucose levels on a low-fat/high-sucrose diet. Once again, there are no reports of this nature in the DBA/2J mice.

More investigations with obesity-prone compared to control strains are clearly needed to identify mechanisms that specifically underlie a propensity toward obesity. They would be greatly strengthened by including more than one control strain that allows immediate confirmation of a given result, testing more than one obesity-prone strain that may reveal differences in mechanisms of obesity, and examining the strains at a young age when they are still similar in body weight. To achieve these objectives, a total of six inbred mouse strains were examined in this study. Three of these, C3H/HeJ, BALB/cByJ and C57L/J, were tested to determine whether they provide stable behavioral and endocrine

measures that are suitable as a control baseline. Three additional strains, AKR/J, DBA/2J and C57BL/6J, known to be obesity-prone were characterized by comparisons to the control strains, as well as to each other. A variety of parameters, including the hypothalamic peptide galanin, were measured to assess whether such comparisons between control and obesity-prone inbred mice yield patterns similar to those detected in lean and obese outbred rats.<sup>17,18</sup> Taking advantage of the fact that inbred strains can be examined at a young age prior to their development of obesity, these mice were introduced into the experiments at 5 weeks of age before showing differential body weight.

## Materials and methods

### Subjects

Six male, inbred mouse strains, C3H/HeJ, BALB/cByJ, C57L/J, C57BL/6J, DBA/2J, AKR/J, were purchased at 4 weeks of age, from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed individually in Plexiglass cages containing bed o-cobs, in a fully accredited AAALAC facility (22°C, with lights off at 1500 for 12 h) according to institutionally approved protocols as specified in the *NIH Guide to the Use and Care of Animals*. Both food and water were available *ad libitum*. All mice were maintained on rodent chow for 1 week before the start of the experiment, were switched to their specific diets for 1 day or 3 weeks, and then killed. At the outset of the experiment, these mouse strains at 5 weeks of age on rodent chow were similar in body weight (18–21 g).

### Diets

Three different feeding paradigms were used, with 1, 2 or 3 diets available *ad libitum*. In the 3-diet choice paradigm, the protein diet (3.7 kcal/g) consisted of 93% casein (Bioserv) mixed with 4% minerals (USP XIV Salt Mixture Briggs, I.C.N. Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification Mixture, I.C.N. Pharmaceuticals) and 0.03% cysteine (L-cysteine hydrochloride, I.C.N. Pharmaceuticals), and the carbohydrate diet (3.7 kcal/g) was composed of 28% dextrin, 28% cornstarch (I.C.N. Pharmaceuticals) and 37% sucrose (Domino) mixed with 4% minerals and 3% vitamins. The fat diet (7.7 kcal/g) consisted of either 86% lard (Armour) or 86% corn oil (Mazola), each mixed with 8% minerals and 6% vitamins. In the 2-diet choice paradigm, a high-fat and a high-carbohydrate diet were presented simultaneously. With diet composition calculated as percentage of total kilocalories, the high-fat diet (4.5 kcal/g) consisted of 25% protein, 25% carbohydrate and 50% fat containing both lard (75%) and corn oil (25%), and the high-carbohydrate diet (3.75 kcal/g) consisted of 25% protein, 65% carbohydrate and 10% fat from corn oil. In the 1-diet paradigm, the high-fat diet with 50% fat was given alone. All diets were presented in separate metal jars, which had a metal top with a small opening (3 cm) that allowed the mice to feed with minimal spillage.

### Experimental procedures

Three experiments were performed with different feeding paradigms, with each experiment examining six mouse strains having  $n=7-8$  mice/strain. In Experiment 1, the mice were given a choice of two diets, high-fat and high-carbohydrate. In Experiment 2, three diets were provided, with the fat diet containing either lard or corn oil, and Experiment 3 tested the mice on a single, high-fat diet with 50% fat. Measurements of food intake, spillage and body weight were taken 3 times per week for the duration of the experiment. Measurements of dietary preferences were calculated as % calories of a specific macronutrient relative to total calories consumed. All mice were killed at the end of the light period (1–2 h before dark onset) by rapid decapitation. Their unilateral body fat from three regions (inguinal, retroperitoneal and epididymal) as well as the mesenteric fat pad, was dissected and weighed, with fat pad weights recorded as a sum of the four individual fat pads. In Experiments 2 and 3, trunk blood was collected for measurements of hormones and metabolites, and brains were rapidly removed and prepared for assay of galanin in the paraventricular nucleus (PVN).

### Hormone and metabolite measures

Serum was analyzed for levels of the hormones, leptin and insulin, using RIA assay kits from Linco Research Inc. (MO, USA). The metabolites, glucose and triglycerides (TG), were measured with an E-Max Microplate Reader using glucose Trinder Reagent Kit or TG Assay Kit, respectively (Sigma, St Louis, MO, USA).

### Brain dissection

Immediately after killing, the brain was placed in a mouse matrix with the ventral surface facing up, and two 0.5 mm coronal sections were made, with the middle optic chiasma as the anterior boundary. The sections were placed on a glass slide, and with the help of a mouse brain atlas,<sup>19</sup> the PVN (bregma  $-0.58$  to  $-0.94$  mm) was rapidly microdissected under a microscope, using the fornix and third ventricle as landmarks. This nucleus was dissected as a reversed isosceles triangle, 0.5 mm bilateral to the ventricle and between the fornix structures, as described.<sup>20</sup> These dissections were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until processed.

### Radioimmunoassay

The microdissected samples of the PVN were expelled into 2.0 M acetic acid, and galanin-like immunoreactivity was measured as described,<sup>17</sup> using polyclonal antisera generated in rabbits to a synthetic rat galanin and rat  $^{125}\text{I}$ -labeled galanin (Peninsula Laboratories). The samples were reconstituted in assay buffer, and the primary antibody was diluted in buffer containing normal rabbit serum (1:75 000

and 0.5% final concentration, respectively). Buffer, antibody, samples, or synthetic rat galanin standards were added at the setup and incubated for 72 h at  $4^{\circ}\text{C}$ . The radiolabeled galanin was then added, and incubation was continued for 24 h. Phase separation was achieved by the addition of goat anti-rabbit  $\gamma$  globulin. This assay has a sensitivity of 4 pg, an  $\text{ED}_{50}$  of 55 pg, and intra- and inter-assay coefficients of variation of 7 and 18%, respectively.

### Real-time quantitative PCR

As previously described,<sup>20</sup> total RNA from pooled micro-dissected PVN samples was extracted with TRIzol reagent. RNA was treated with RNase-free DNase I to remove any contaminating genomic DNA before RT. cDNA and minus RT were synthesized using an oligo-dT primer with or without SuperScript II Reverse Transcriptase. The SYBR Green PCR core reagents kit (Applied Biosystems, CA, USA) was used, with  $\beta$ -actin as endogenous control. PCR was performed in MicroAmp Optic 96-well Reaction Plates (Applied Biosystems) on an ABI PRISM 7700 Sequence Detection system (Applied Biosystems), with the condition of 2 min at  $50^{\circ}\text{C}$ , 10 min at  $95^{\circ}\text{C}$ , then 40 cycles of 15 s at  $95^{\circ}\text{C}$  and 1 min at  $60^{\circ}\text{C}$ . Each study consisted of four independent runs of PCR in triplicate, and each run included a standard curve, nontemplate control, and negative RT control. The levels of galanin gene expression were quantified relative to the level of  $\beta$ -actin, using standard curve method. The primers, designed with ABI Primer Express V.1.5a software based on published sequences, were: (1)  $\beta$ -actin: 5'-GGCCAACCGT GAAAAGATGA-3' (forward) and 5'-CACAGCCTGGATGGC TACGT-3' (reverse); and (2) galanin: 5'-TTCCCACCCTGCT CAAGATG-3' (forward) and 5'-TGGCTGACAGGGTTGCAA-3' (reverse). The concentrations of primers were 100–200 nM, and all reagents, unless indicated, were from Invitrogen.

### Data analysis

Caloric intake was averaged over the 3-week test period and data presented as kilocalories/24 h. The measures of body weight and fat pad weights were recorded on the final day of the experiment. All values are expressed as mean  $\pm$  s.e.m. With a standard statistical package (SPSS), data were analyzed using a one-way ANOVA, and if statistically significant at  $P < 0.05$ , this analysis was followed by a Bonferroni *post hoc* test for multiple comparisons between groups. Within group measures of macronutrient intake and fat pad weights were correlated using a Pearson's product moment correlation.

## Results

Three experiments were performed with different feeding paradigms and various measures of eating behavior, body weight, circulating hormones and metabolites and galanin in the PVN.

**Experiment 1: macronutrient preference on a 2-diet feeding paradigm**

This experiment characterized and compared the three control and three obesity-prone strains with respect to their macronutrient preferences and weight gain in a 2-diet feeding paradigm. The six strains were maintained for 3 weeks on both a high-carbohydrate (65% carbohydrate, 10% fat) and a high-fat (50% fat, 25% carbohydrate) diet, each with 25% protein. After 3 weeks on the two diets, the control and obesity-prone DBA/2J and C57BL/6J strains were still comparable to the controls in body weight (23–26 g) and daily weight gain (0.15–0.25 g/day), but the AKR/J strain became significantly heavier compared to all other strains (Table 1). The weights of the four dissected fat pads of the control strains fell within a tight range of 0.52–0.62 g. Compared to this consistent baseline, the obesity-prone strains each had significantly heavier fat depots, with the C57BL/6J score significantly lower than the AKR/J and DBA/2J (Table 1).

This increase in adiposity, occurring with a similar total caloric intake across the six strains (19–22 kcals/day), may be related to the differences in their diet intake patterns (Table 1). The three control strains were very similar to each other in selecting a balanced diet, with 50% carbohydrate and 25% fat. Compared to this baseline, the obesity-prone strains each ate significantly more fat and less carbohydrate (Table 1). The AKR/J and DBA/2J mice consumed the greatest amount of fat (38–42%), significantly more than the control mice (22–26%,  $P < 0.001$ ) in addition to the C57BL/6J strain (34%,  $P < 0.05$ ) (Table 1). Thus, preference for dietary fat increased in direct proportion to body mass. These results, showing a stable baseline for the three control strains, reveal clear differences between the obesity-prone and control strains and hint at possible differences between the C57BL/6J mice and the other obesity-prone strains.

**Experiment 2: macronutrient preference, hormones and PVN galanin on a 3-diet feeding paradigm**

To confirm the eating patterns of Experiment 1 and obtain additional information on endocrine and hypothalamic processes, this experiment examined the control and

obesity-prone mouse strains in a 3-diet feeding paradigm. Two sets of inbred mice were maintained for 3 weeks on the three pure macronutrient diets, with the fat diet consisting of lard in one set and corn oil in the other. Since very similar results were obtained for these two sets of mice, indicating that the type of fat source is not critical, only the data for the lard group are presented here.

Consistent with the 2-diet paradigm of Experiment 1, the results for the 3-diet paradigm distinguished the AKR/J and DBA/2J mice as having the greatest body fat and strongest preference for fat and differentiated these strains from the C57BL/6J mice in several respects. Once again, the 6 mouse strains were similar in body weight at the beginning and end of the experiment at 5 weeks of age, with only the AKR/J strain showing a significant difference after 3 weeks on the macronutrient diets (Table 2). Compared to the control strains, all three obesity-prone strains had heavier fat pad weights, although the C57BL/6J score was significantly lower than the AKR/J and DBA/2J ( $P < 0.05$ ). While total caloric intake was similar across strains, this 3-diet feeding paradigm revealed distinct differences between the control and obesity-prone mice in their patterns of macronutrient intake (Table 2). As in Experiment 1, the control strains were similar to each other in showing a strong preference for carbohydrate (60%), relative to fat (15%) and protein (25%). Also, the obesity-prone mice differed markedly from these controls, revealing a significantly weaker preference for carbohydrate (30–40%) and stronger preference for fat, which tended to be higher in the AKR/J (38%) and DBA/2J (36%) compared to the C57BL/6J (32%). A *post hoc* analysis of the relationship between absolute fat intake and fat pad weights revealed strong, positive correlations for both the AKR/J ( $r = +0.60$ ,  $P < 0.01$ ) and DBA/2J ( $r = +0.70$ ,  $P < 0.001$ ) mice, indicating that the higher fat calories may contribute to the larger fat depots. This relationship was considerably weaker for the C57BL/6J mice ( $r = +0.45$ ,  $P < 0.05$ ) and not evident in the control strains, which showed insignificant inverse correlations.

The measure of protein preference in this 3-diet paradigm revealed a distinct pattern for the C57BL/6J strain. Although the controls and two obesity-prone AKR/J and DBA/2J strains

**Table 1** Measures on a 2-diet feeding paradigm (Experiment 1)

Strain	n	Body weight (g)	Body fat (g)	Total (kcal)	Fat (kcal)	Carbohydrate (kcal)	Fat preference (%)
<i>Control</i>							
C3H/HeJ	7	24 ± 1.1	0.62 ± 0.08	21 ± 1.7	5.4 ± 0.4	10.2 ± 1.2	26 ± 3.1
BALB/cByJ	7	23 ± 0.8	0.52 ± 0.10	21 ± 1.5	5.0 ± 0.5	10.6 ± 1.1	21 ± 1.8
C57L/J	7	25 ± 1.0	0.58 ± 0.12	21 ± 1.1	5.5 ± 0.4	10.2 ± 0.8	26 ± 2.2
<i>Obesity-prone</i>							
C57BL/6J	7	26 ± 0.7	1.06 ± 0.15 <sup>b</sup>	19 ± 1.0	6.6 ± 0.4 <sup>b</sup>	7.5 ± 1.1 <sup>a</sup>	35 ± 2.1
DBA/2J	7	26 ± 1.2	1.45 ± 0.08 <sup>a</sup>	22 ± 1.0	8.3 ± 0.5 <sup>a</sup>	7.9 ± 0.6 <sup>a</sup>	39 ± 3.9
AKR/J	7	32 ± 1.0 <sup>b</sup>	1.62 ± 0.09 <sup>a</sup>	22 ± 0.4	9.3 ± 0.2 <sup>a</sup>	7.5 ± 0.4 <sup>a</sup>	43 ± 1.7

Given are mean ± s.e.m. One-way ANOVA across strains showed significant differences ( $P < 0.05$ ) for all measures except total kilocalorie intake. All strains had similar body weights (18–21 g) at the beginning of the experiment. Intake scores reflect kilocalories consumed in 24 h. Fat preference indicates % fat calories relative to total calories consumed in 24 h. <sup>a</sup> $P < 0.05$  compared to each of lean strains; <sup>b</sup> $P < 0.05$  compared to all other strains.

had a similar range of protein preference (20–30%), the C57BL/6J mice exhibited significantly higher protein intake (Table 2) and stronger preference for this macronutrient (41%,  $P < 0.01$ ) compared to all other strains. This finding was similarly detected in the second set of mice tested with the corn oil as the fat source. In this group, the C57BL/6J mice consumed  $8.0 \pm 0.4$  kcals/day of protein, which was significantly greater ( $P < 0.05$ ) than that of the other five strains (4.1–5.6 kcals/day).

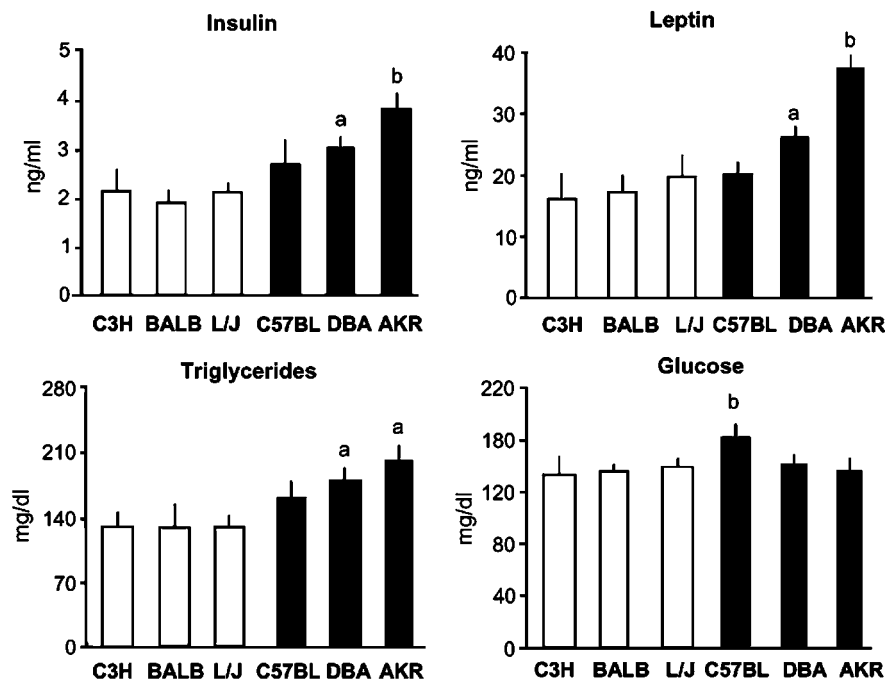
Additional measures in these mice on the 3-diet paradigm with lard further identified differences between the obesity-prone and control strains and also between the C57BL/6J and the AKR/J and DBA/2J strains. The three control strains

showed a tight range of scores for insulin, leptin, TG and glucose (Figure 1). Compared to this consistent baseline, the fat-preferring AKR/J and DBA/2J mice had significantly higher levels of insulin and leptin, a difference not seen in the C57BL/6J mice (Figure 1). The TG levels were also higher in the AKR/J and DBA/2J but only slightly elevated in the C57BL/6J mice. In contrast, the glucose levels were significantly elevated in the C57BL/6J compared to all other strains. These endocrine patterns were accompanied by strain differences in galanin peptide measured via RIA. Compared to the stable baseline for the control strains, the AKR/J and DBA/J showed significantly higher galanin levels in the PVN, elevated by almost 200% (Figure 2). With only a

**Table 2** Measures on a 3-diet feeding paradigm (Experiment 2)

Strain	n	Body weight (g)	Body fat (g)	Total (kcals)	Fat (kcals)	Carbohydrate (kcals)	Protein (kcals)
<i>Control</i>							
C3H/HeJ	8	$23 \pm 0.7$	$0.65 \pm 0.05$	$17 \pm 1.6$	$2.1 \pm 0.2$	$10.3 \pm 1.2$	$5.0 \pm 0.7$
BALB/cByJ	8	$23 \pm 1.1$	$0.63 \pm 0.04$	$18 \pm 1.4$	$2.3 \pm 0.2$	$10.8 \pm 1.5$	$4.8 \pm 0.7$
C57L/J	8	$24 \pm 0.6$	$0.79 \pm 0.15$	$18 \pm 1.5$	$3.1 \pm 0.1$	$11.3 \pm 1.2$	$4.3 \pm 0.5$
<i>Obesity-prone</i>							
C57BL6/J	8	$25 \pm 0.4$	$1.18 \pm 0.12^b$	$20 \pm 1.0$	$6.5 \pm 0.9^a$	$5.7 \pm 0.9^a$	$8.2 \pm 0.6^b$
DBA/2J	8	$25 \pm 0.9$	$1.57 \pm 0.20^a$	$19 \pm 1.1$	$6.9 \pm 1.1^a$	$6.2 \pm 0.9^a$	$6.0 \pm 0.8$
AKR/J	8	$30 \pm 1.0^b$	$1.89 \pm 0.12^a$	$19 \pm 0.9$	$7.3 \pm 0.9^a$	$7.9 \pm 1.1^a$	$4.1 \pm 0.3$

Given are mean  $\pm$  s.e.m. One-way ANOVA across strains showed significant differences ( $P < 0.05$ ) for all measures except total kilocalorie intake. All strains had similar body weights (18–21 g) at the beginning of the experiment. Intake scores reflect kilocalories consumed in 24 h. <sup>a</sup> $P < 0.05$  compared to each of lean strains; <sup>b</sup> $P < 0.05$  compared to all other strains.



**Figure 1** Endocrine measures for the three control (C3H/HeJ, BALB/cByJ, C57L/J) and three obesity-prone (C57BL/6J, DBA/2J, AKR/J) strains ( $n = 8$ /strain) maintained for 3 weeks on a 3-diet macronutrient paradigm of carbohydrate, protein and fat (Experiment 2). Values represent the mean of the group  $\pm$  s.e.m. One-way ANOVA revealed significant strain differences ( $P < 0.05$ ) for each of the measures of insulin, leptin, triglycerides and glucose. a:  $P < 0.05$  for direct comparisons between the obesity-prone and control strains; b:  $P < 0.05$  for direct comparisons to all other strains.

small increase in the C57BL/6J mice (30%), PVN galanin levels remained significantly lower than that of the other obesity-prone strains ( $P < 0.01$ ).

*Experiment 3: hormones and PVN galanin expression on a 1-diet feeding paradigm with 50% fat*

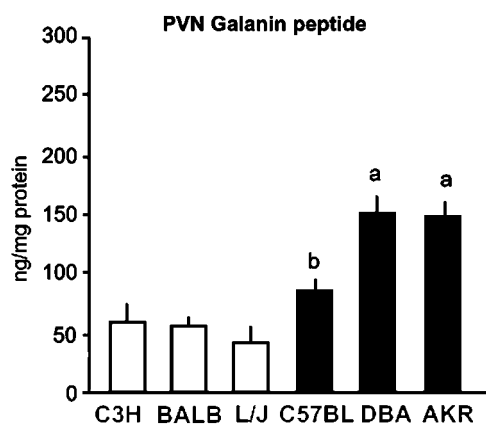
To assess whether these endocrine and peptide changes can occur independently of the higher fat intake of the obesity-prone mice, this experiment tested an additional set of three control and three obesity-prone strains, which were maintained for 1 day on a single high-fat diet with 50% fat. In these mice, PVN galanin was examined through measurements of gene expression using real-time quantitative PCR.

As in Experiments 1 and 2, these strains were similar in their total caloric intake and also in their body weights at the beginning and end of the experiment, with the exception of

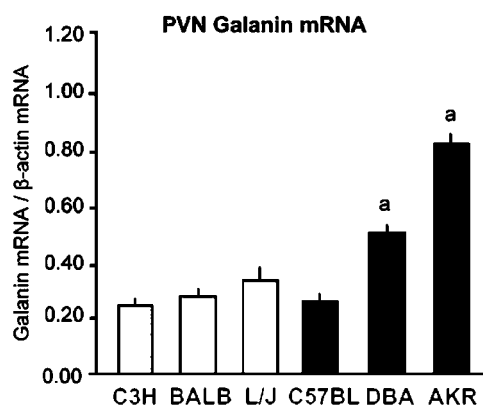
the AKR/J strain (Table 3). The measures of fat pad weights, TG and leptin yielded significantly higher scores for the AKR/J and DBA/2J mice, but not the C57BL/6J, compared to the control strains. Consistent with Experiment 2, this pattern was accompanied by a difference in galanin gene expression, indicated by the ratio of peptide mRNA/ $\beta$ -actin mRNA (Figure 3). Galanin expression in the PVN was significantly elevated in the AKR/J and DBA/2J strains, compared to the control mice as well as C57BL/6J mice, which showed no difference from controls.

**Discussion**

These experiments in inbred mice, at 5–8 weeks of age, revealed behavioral, endocrine and peptide measures that were clearly different between the obesity-prone and control



**Figure 2** Six inbred strains ( $n = 8$ /strain) distinguished by their galanin peptide levels in the PVN on a 3-diet feeding paradigm (Experiment 2). Values represent the mean of the group  $\pm$  s.e.m. The three obesity-prone strains (C57BL/6J, DBA/2J, AKR/J) had significantly higher galanin levels compared to the control strains (C3H/HeJ, BALB/cBy, C57L/J) ( $F(5,42) = 7.88$ ,  $P < 0.001$ ), with the AKR/J and DBA/2J showing a much larger difference (+200%) than the C57BL/6J (+30%). a:  $P < 0.05$  for direct comparisons between the obesity-prone and control strains; b:  $P < 0.05$  for direct comparisons between the C57BL/6J and all other strains.



**Figure 3** Six inbred strains ( $n = 7$ /strain) distinguished by their galanin expression levels (galanin mRNA/ $\beta$ -actin mRNA) in the PVN on a 1-diet feeding paradigm with 50% fat (Experiment 3). Values represent the mean  $\pm$  s.e.m., yielding a significant one-way ANOVA across strains ( $F(5,30) = 48.93$ ,  $P < 0.001$ ). Direct comparisons between the strains revealed significantly higher galanin expression in the obesity-prone AKR/J and DBA/2J but not the C57BL/6J compared to control strains (C3H/HeJ, BALB/cBy, C57L/J). a:  $P < 0.05$  for direct comparisons between the obesity-prone AKR/J or DBA/2J mice and all other strains.

**Table 3** Measures on a high-fat diet feeding paradigm (Experiment 3)

Strain	n	Body weight (g)	Body fat (g)	Total (kcal)	Triglycerides (mg/dl)	Leptin (ng/ml)
<i>Control</i>						
C3H/HeJ	8	24 $\pm$ 0.5	0.64 $\pm$ 0.05	18 $\pm$ 2.1	122 $\pm$ 14	21.6 $\pm$ 2.3
BALB/cByJ	8	25 $\pm$ 1.1	0.65 $\pm$ 0.04	20 $\pm$ 1.8	125 $\pm$ 10	22.6 $\pm$ 0.9
C57L/J	8	24 $\pm$ 0.6	0.52 $\pm$ 0.15	21 $\pm$ 1.4	ND	24.1 $\pm$ 5.5
<i>Obesity-prone</i>						
C57BL6/J	8	24 $\pm$ 0.4	0.48 $\pm$ 0.12	22 $\pm$ 1.5	132 $\pm$ 16	22.9 $\pm$ 2.1
DBA/2J	8	24 $\pm$ 0.9	1.05 $\pm$ 0.10 <sup>a</sup>	22 $\pm$ 1.3	167 $\pm$ 15 <sup>a</sup>	31.0 $\pm$ 1.1 <sup>a</sup>
AKR/J	8	30 $\pm$ 1.0 <sup>b</sup>	1.25 $\pm$ 0.12 <sup>a</sup>	20 $\pm$ 1.0	200 $\pm$ 16 <sup>a</sup>	30.7 $\pm$ 3.4 <sup>a</sup>

Given are mean  $\pm$  s.e.m. One-way ANOVA across strains showed significant differences ( $P < 0.05$ ) for all measures except total kilocalorie intake. All strains had similar body weights (18–21 g) at the beginning of the experiment. Total intake scores reflect kilocalories consumed in 24 h. <sup>a</sup> $P < 0.05$  compared to each of lean strains and C57BL/6J; <sup>b</sup> $P < 0.05$  compared to all other strains.

strains and also between the C57BL/6J and the other obesity-prone mice.

The inbred strains used for the control baseline, C3H/HeJ, BALB/cByJ and C57L/J, were selected based on earlier reports showing that these three strains gain only moderately on a high-calorie diet, relative to the higher vs lower weight gain of the obesity-prone vs obesity-resistant mice, respectively.<sup>1–3</sup> They were also chosen based on other evidence showing the BALB/cByJ and C57L/J strains to prefer carbohydrate over fat when given a choice of macronutrients.<sup>3,7</sup> In the different feeding paradigms tested here, the present results confirm these behavioral patterns in the BALB/cByJ and C57L/J strains. They further demonstrate a similar pattern in the C3H/HeJ mice and reveal a close range of scores for all three strains that support their use as a stable, control baseline. After 3 weeks on the different diets, these control strains were similar to each other in their measures of total caloric intake (18–22 kcal/day) and body weights (23–25 g) at 8 weeks of age. Further, with a choice of macronutrients in the 2-diet and 3-diet feeding paradigms, these control strains each selected a balanced diet consisting of 50–60% carbohydrate, 15–25% fat and 25% protein. They also had similar scores for fat pad weights, as well as for levels of insulin (1.9–2.2 ng/ml), leptin (17–22 ng/ml), TG (120–140 mg/dl) and glucose (140–150 mg/dl) on the different diets. These results demonstrate, for the first time, a consistency between these three strains in their endocrine as well as behavioral scores, which should provide a suitable baseline for comparisons with the different obesity-prone strains.

Comparisons to the control strains revealed distinct behavioral patterns in the AKR/J, DBA/2J and C57BL/6J mice known to be susceptible to the obesity-promoting effects of a high-fat diet.<sup>1,7–9,21</sup> These differences were seen even when the obesity-prone strains were similar to controls in their body weight, both at the beginning of the experiment (5 weeks of age) when maintained on lab chow and also at the end of the experiment (8 weeks of age) after only 1 day on the high-fat diet. The measure of dissected fat pad weights revealed a significant increase in adiposity in all obesity-prone strains. In the 2-diet and 3-diet feeding paradigms, this was accompanied by a stronger preference for dietary fat (30–40%) compared to the control baseline (15–25%), with carbohydrate intake shifting in the reverse direction. This confirms and extends with further comparisons the results of two previous studies that compared the AKR/J to C57L/J<sup>7</sup> or obesity-prone strains to C3H/HeJ and BALB/cByJ.<sup>3</sup> Of particular note in the present study is the finding that fat intake and body fat were strongly, positively correlated in the AKR/J and DBA/2J mice but not in the control strains, which had a narrower range of scores showing a slight inverse relationship. This finding, previously described in the AKR/J strain,<sup>3</sup> gives further suggestion of differences between the control and obesity-prone strains in their mechanisms controlling fat ingestion and deposition.

A further difference in the magnitude of their response relative to controls was also detected in the C57BL/6J mice

compared to the other obesity-prone strains. In each of the three experiments, the scores for the fat pad weights of the C57BL/6J were consistently lower than those of the AKR/J and DBA/2J strains. This confirms and extends previous studies comparing the C57BL/6J strain to the DBA/2J in one report<sup>6</sup> or to the AKR/J in another.<sup>3</sup> The scores for fat intake and fat preference (32–34%) of the C57BL/6J were also consistently lower compared to the other obesity-prone strains (36–42%), as was the correlation between fat intake and adiposity. In addition, a distinct characteristic of the C57BL/6J was detected in their measures of protein intake and preference (40%), which were significantly greater compared to all other strains (20–30%). Thus, the present results show how the C57BL/6J strain, while clearly obesity-prone with a preference for fat over carbohydrate, differs from the AKR/J and DBA/2J strains in its behavioral profile and, thus, possibly in its mechanisms underlying obesity.

Along with their stronger fat preference, the obesity-prone strains also exhibited a distinct endocrine profile, which once again was similar for the AKR/J and DBA/2J but different for the C57BL/6J. Compared to the baseline scores of the control mice, the AKR/J and DBA/2J mice in the different feeding paradigms had elevated levels of insulin and leptin as well as higher TG levels but showed no difference in glucose. While prior studies comparing obesity-prone to obesity-resistant mice have revealed higher levels of insulin and glucose in the prone mice on a high-fat diet,<sup>11,13</sup> there is only one report that has directly compared the obesity-prone to a control strain and showed the AKR/J (vs C57L/J) to exhibit elevated leptin and TG on a high-fat diet.<sup>7</sup> This endocrine profile, clearly similar to that seen in obesity-prone rats and humans compared to lean subjects,<sup>7,22–24</sup> substantiates the idea that the control mice used here for comparison provide a reasonable baseline for revealing strain differences. Once again, the C57BL/6J strain showed no significant differences in insulin, leptin and TG compared to the control mice and had significantly lower levels than the AKR/J and DBA/2J mice. Thus, although the C57BL/6J strain develops hyperinsulinemia and hyperleptinemia on a high-fat diet compared to a low-fat diet<sup>5,11,13,25</sup> and in comparisons to a leaner, obesity-resistant strain,<sup>4,5</sup> such differences are not evident with comparisons to the control strains of comparable body weight. This is supported by two other studies, which revealed significantly lower leptin in the C57BL/6J compared to DBA/2J mice<sup>6</sup> and lower insulin and TG compared to AKR/J mice.<sup>15</sup> A further difference in the C57BL/6J mice was detected with the measure of glucose. In the 3-diet feeding paradigm, this strain exhibited hyperglycemia compared to the control strains as well as the obesity-prone strains. This was seen in another study comparing the C57BL/6J to AKR/J mice on a single, high-fat diet.<sup>15</sup> Thus, in addition to their behavioral patterns, the endocrine profile of C57BL/6J mice clearly differs from that of the other obesity-prone strains, again suggesting differences in their physiological systems.

There is limited information in the literature on hypothalamic peptides and their role in controlling feeding and body weight in obesity-prone inbred mice. Studies that exist have generally measured NPY or agouti-related protein in the arcuate nucleus and have examined the effect of a high-fat diet compared to a low-fat diet on the expression of these peptides. The results obtained have been mixed. Whereas some studies describe a suppressive effect in C57BL/6J mice in specific experimental paradigms,<sup>26–29</sup> others reveal this effect only in obesity-resistant mice but not in the C57BL/6J strain,<sup>30,31</sup> while the opposite effect, a high-fat diet-induced stimulation of NPY, is described in the DBA/2J mice.<sup>6</sup> There is only one study that has actually made direct comparisons between these different strains in their hypothalamic peptides. In this report, the obesity-prone AKR/J strain compared to the control C57L/J mice had significantly elevated expression of the feeding-stimulatory, orexin peptides in the perifornical lateral hypothalamus.<sup>7</sup> Of particular note is that this increase in orexin expression is associated with elevated TG levels, which are known to promote expression of the orexins.<sup>7,20</sup>

The results obtained here with measurements of galanin in the PVN provide further evidence for disturbances similarly related to circulating TG. Compared to the baseline values of each of the control strains, PVN galanin peptide levels and gene expression were significantly higher in the AKR/J and DBA/2J mice. This peptide difference in these two strains, accompanied by increased fat intake and adiposity, confirms in these obesity-prone mice the evidence obtained in outbred Sprague–Dawley rats in relation to dietary fat.<sup>17,32–36</sup> The rise in TG observed in the AKR/J and DBA/2J mice further substantiates these rat studies, which demonstrate a close relationship between PVN galanin and circulating lipids.<sup>18</sup> This relationship is evident under natural feeding conditions and also after injection of intralipid, which raises circulating TG while stimulating galanin and the orexin peptides.<sup>7,20,32</sup> The closeness of this link between TG and PVN galanin is evident in the additional result showing both measures to remain stable or marginally elevated in the C57BL/6J mice. Although the higher galanin peptide on the 3-diet paradigm of the AKR/J and DBA/2J mice may be attributed in part to their increased fat intake as reported in rats,<sup>32</sup> the AKR/J and DBA/2J mice on a single high-fat diet both showed significantly elevated galanin expression even though their total caloric intake was equal to controls. This new finding suggests that the endogenous PVN galanin system in these obesity-prone mice is more sensitive to a given calorie of fat consumed, with this greater sensitivity translated into a stronger effector response. This is supported by evidence that the effects induced by galanin, a stimulation of food intake, body weight and body fat accrual, are stronger in animals consuming a fat-rich diet.<sup>18,37–41</sup> Galanin on a high-fat diet may contribute to the higher weight gain of these obesity-prone mice through various metabolic effects, including a reduction in energy expenditure and sympathetic nervous system activity.<sup>42,43</sup>

The results obtained in the C57BL/6J mice, which differ from the AKR/J and DBA/2J, argue against a major role for galanin and circulating lipids in this strain. While showing greater fat intake compared to the control mice, this difference was consistently smaller than that seen in the other obesity-prone strains, as was their correlation between fat intake and size of the fat depots. Also, the C57BL/6J strain compared to controls showed no difference or marginally higher levels of PVN galanin expression or peptide levels, even though their fat intake was somewhat elevated, and their TG levels were similar to controls as well as significantly lower than the other obesity-prone strains. This new evidence suggests that the mechanisms underlying the propensity of the C57BL/6J strain toward obesity differ from those of the AKR/J and DBA/2J strains in being less closely related to dietary fat, circulating lipids and PVN galanin. While there are no other studies directly comparing the C57BL/6J to a set of control mice, there is evidence showing this strain to be different from the AKR/J and DBA/2J in their measures of glucose homeostasis. The C57BL/6J mice exhibit greater hyperglycemia and gluconeogenesis on a high-fat diet.<sup>6,15</sup> A similar result was observed here in the 3-diet feeding paradigm, which revealed elevated glucose levels in the C57BL/6J relative to the other obesity-prone strains. Whereas the mechanisms underlying these disturbances in glucose homeostasis remain to be defined, they may be related in some way to the unusually strong preference of the C57BL/6J mice for protein, along with their lower fat preference and TG levels, compared to the other obesity-prone strains. Consumption of a high-protein diet is known to increase glucose levels while reducing TG<sup>44–46</sup>

## Conclusions

The results of this present study reveal stable baseline scores for the control strains, C3H/HeJ, BALB/cByJ and C57L/J. Relative to this baseline, they also provide evidence for a phenotype in the obesity-prone AKR/J and DBA/2J strains, characterized by elevated fat intake, TG levels and PVN galanin, and a somewhat different profile in the C57BL/6J strain, indicated by elevated protein intake and hyperglycemia. These phenotypes are evident at a young age, sometime before 8 weeks of age. They also occur in the absence of hyperphagia and in some cases prior to significant changes in body weight. This suggests that the mechanisms reflected by these differences may have a causal role in the differential body fat accrual exhibited by these strains. Further studies of these inbred mice relative to controls, at even younger ages, may be helpful in revealing subtle disturbances that are critical to their differential propensities toward obesity. Such studies should include measurements of other peptide systems, besides galanin, that are likely to contribute to an increased propensity toward obesity.

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