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Behavioral, physiological, and molecular differences in response to dietary restriction in three inbred mouse strains

Cigdem Gelegen,[1,2] David A. Collier,[2] Iain C. Campbell,[3] Hugo Oppelaar,[1] and Martien J. H. Kas[1]

[1]*Department of Pharmacology and Anatomy, Behavioural Genomics Section, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Utrecht, The Netherlands; King's College Institute of* [3]*Psychiatry and* [2]*Social, Genetic, and Developmental Psychiatry Research Centre, London, United Kingdom*

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Gelegen, Cigdem, David A. Collier, Iain C. Campbell, Hugo Oppelaar, and Martien J. H. Kas. Behavioral, physiological, and molecular differences in response to dietary restriction in three inbred mouse strains. *Am J Physiol Endocrinol Metab* 291: E574–E581, 2006. First published May 2, 2006; doi:10.1152/ajpendo.00068.2006.—Food restriction paradigms are widely used in animal studies to investigate systems involved in energy regulation. We have observed behavioral, physiological, and molecular differences in response to food restriction in three inbred mouse strains, C57BL/6J, A/J, and DBA/2J. These are the progenitors of chromosome substitution and recombinant inbred mouse strains used for mapping complex traits. DBA/2J and A/J mice increased their locomotor activity during food restriction, and both displayed a decrease in body temperature, but the decrease was significantly larger in DBA/2J compared with A/J mice. C57BL/6J mice did not increase their locomotor activity and displayed a large decrease in their body temperature. The large decline in body temperature during food restriction in DBA/2J and C57BL/6J strains was associated with a robust reduction in plasma leptin levels. DBA/2J mice showed a marked decrease in white and brown adipose tissue masses and an upregulation of the antithermogenic hypothalamic neuropeptide Y Y_1 receptor. In contrast, A/J mice showed a reduction in body temperature to a lesser extent that may be explained by downregulation of the thermogenic melanocortin 3 receptor and by behavioral thermoregulation as a consequence of their increased locomotor activity. These data indicate that genetic background is an important parameter in controlling an animal's adaptation strategy in response to food restriction. Therefore, mouse genetic mapping populations based on these progenitor lines are highly valuable for investigating mechanisms underlying strain-dependent differences in behavioral physiology that are seen during reduced food availability.

locomotor activity; body temperature; food intake; neuropeptide Y; melanocortin

ENERGY BALANCE is regulated by processes that influence food intake and energy expenditure. The main components of energy expenditure are metabolism and thermogenesis induced by exercise, cold, and diet, and these are regulated by the interaction of behavioral, physiological, and molecular mechanisms. Imbalances in energy state can result in health problems, such as malnutrition, eating disorders, or obesity (10, 27, 41, 46, 59).

Food restriction paradigms are widely used in animal studies to investigate mechanisms involved in the regulation of energy balance (14, 16, 20, 38, 58). In endothermic organisms, food restriction is associated with a decrease in body temperature (12, 28, 44, 60); this is thought to conserve energy by reducing resting metabolic rate (12, 12, 54). In rats, starvation-induced hypothermia is the result of a decrease in the threshold temperature that activates thermogenic systems; different adaptation strategies are used to counteract an excessive fall in body temperature (47). In different inbred mouse strains, changes in mean body temperature in response to food restriction vary significantly (43), and this variation can be used to investigate the molecular determinants associated with different responses to food restriction.

Behavioral thermoregulation is one of the mechanisms by which endothermic animals achieve and maintain a stable body temperature during times of food shortage (12, 47, 54). Increased locomotor activity is a component of behavioral thermoregulation, and the amplitude of the increase may be modified by the degree of restriction. This is suggested by the observation that reduced food intake increases running wheel activity in rodents (24, 30, 33). This phenomenon, which is called activity-based anorexia (ABA), has been proposed as a model for investigating behavioral traits related to anorexia nervosa (24, 45). Rats increase their locomotor activity in response to 3 days of food deprivation (47), and, similarly, an increase in dark- and light-phase locomotor activity is seen in mice exposed to caloric restriction (7, 38, 58). This increased locomotor activity could be a manifestation of food foraging behavior or a means of maintaining core body temperature through exercise-induced thermogenesis.

In addition to behavioral thermogenesis, endothermic animals use autonomic mechanisms to regulate their core temperature (47). For example, brown adipose tissue (BAT)-mediated nonshivering thermogenesis is involved in heat production when animals are exposed to cold. Mitochondrial uncoupling proteins (UCPs) in the BAT generate heat by uncoupling oxidative phosphorylation, and, in mice, targeted inactivation of the gene coding for UCP1 leads to cold sensitivity (13). Leptin increases the thermogenesis in BAT by increasing UCP1 expression (48); therefore, decreased heat production resulting from hypoleptinemia could be associated with the increased locomotor activity seen in some inbred mice strains and rats in response to restricted feeding. Leptin's effects on the hypothalamic neuropeptide Y (NPY) and melanocortin systems have been implicated in the regulation of energy balance (56, 59). For example, selective NPY Y_1 and Y_5 receptor agonists increase food consumption and decrease circulating levels of thyroid hormones, showing that both receptors mediate the stimulatory effects of NPY on food

Address for reprint requests and other correspondence: M. J. H. Kas, Behavioural Genomics Section, Rudolf Magnus Institute of Neuroscience, Univ. Medical Centre Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands (e-mail: m.j.h.kas@med.uu.nl).

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consumption and the inhibitory effects on the hypothalamic-pituitary-thyroid axis (15). On the other hand, melanocortin 3 receptor (MC3R)-deficient mice are hyperleptinemic and have increased fat mass, reduced lean mass, and higher feed efficiency than wild-type littermates, despite being hypophagic and maintaining normal metabolic rates, indicating an important role for this receptor in the regulation of energy homeostasis (6). Furthermore, central infusion of agouti-related protein (an inverse agonist of MC3/MC4 receptors) in rats exposed to ABA counteracts the deregulation of body temperature (33).

Mice provide a useful animal model for studying mechanisms involved in the response to caloric restriction and the regulation of the energy balance. The use of different inbred strains offers the opportunity to elucidate the genetic basis of observed traits. These inbred mouse strains are the progenitors of chromosome substitution strains (CSS) and recombinant inbred strains (RIS), which provide a permanent resource for studying the genetic control of phenotypic variation (50, 57). C57BL/6J, A/J, and DBA/2J inbred strains, which form the genetic background of currently available CSS and one of the RIS panels, show variations in several physiological and behavioral traits (18, 19, 50, 55). In this study, we aimed to determine behavioral, physiological, and genetic differences in C57BL/6J, A/J, and DBA/2J inbred mouse strains to a restricted feeding schedule to investigate the adaptive mechanisms that have developed to counteract changes in food availability.

MATERIALS AND METHODS

Animals. Male C57BL/6J ($n = 16$), DBA/2J ($n = 16$), and A/J ($n = 16$) mice were used. Initial breeding pairs for each strain were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were bred in the Rudolf Magnus Institute of Neuroscience animal facility and were 4–6 mo old at the start of the experiment. After being weaned at 3–4 wk, female and male mice were housed separately in cages (2–4 animals/cage; Macrolon type II; no. 1284 L) in a room maintained on a 12:12-h dark-light cycle (lights on at 2:00 A.M.) and an ambient temperature of 22.0 ± 2°C. They were given unrestricted access to a Special Diets Services (SDS) diet and water. This diet contained 3.4% oil, 18.8% protein, 60.3% carbohydrate, and 3.7% fiber, in addition to 3% minerals, vitamins, and amino acids (SDS; Witham, Essex, UK).

Surgical procedures. Anesthesia was performed using isoflurane in a mixture of N_2O-O_2, with an oxygen concentration of 40–45% during the induction period and the surgical procedure. Anesthesia was induced by inhalation of 5% vaporized isoflurane for 1–2 min in an induction chamber, animals were taken out of the chamber, and during the surgical procedure 1.5% isoflurane was administered through a tube surrounding the nose. After reaching deep anesthesia, transmitters (TA10TA-F20; Data Sciences International, St. Paul, MN) were implanted intraperitoneally for telemetric monitoring of locomotion and body temperature. At the end of the procedure, 0.02 ml/mouse of Temgesic (0.03 mg/ml) was injected subcutaneously for postoperative pain relief. The surgical procedure took 20 min/animal.

Experimental procedures. After 10 days of recovery from surgery, mice from each strain were divided in two groups. The first group was individually housed for 1 wk. This group of mice was defined as "baseline animals." Under baseline conditions, mice had unrestricted access to food and water. Body weight and food intake were measured daily just before the beginning of the dark phase. To continuously collect body temperature and locomotion data (i.e., recording every 10 min), each mouse's cage was placed on a receiver plate (RPC-I; Data Sciences International). The data collected through the transmitter were sent to the receiver plate and were then analyzed using the Dataquest A.R.T data acquisition system (version 1.10; Data Sciences International). A second group of mice from each strain had 1 wk of unrestricted access to food and water, followed by a restricted feeding schedule for five consecutive days (2 h of daily access to food), and this group was defined as "restriction animals." During the restriction period, the food was available ad libitum during the first 2 h of the dark phase (the habitual activity phase of this nocturnal species). Body weight and food intake were measured before and after food access, and body temperature and locomotion data were registered continuously using the same telemetry system. At the end of the restriction period, mice were decapitated in the fasted state during the last hour of the light phase, which is 1 h before the start of scheduled food access. This time point was selected to exclude a possible strain-dependent effect of food intake on gene expression and plasma leptin levels. To control for circadian effect on these measurements in ad libitum control mice, these (baseline) animals were decapitated at the same circadian phase of the light-dark cycle (1 h before the start of the dark phase, their habitual activity phase). To further minimize gene expression and plasma leptin differences between the three strains in relation to the time of decapitation, individual mice from the three strains were decapitated in a random order. In this way, randomized decapitation across strains in the last hour of the light phase prevented the possible introduction of systemic errors that could lead to false significant results in gene expression and plasma leptin analysis across strains under the two experimental conditions. After decapitation, the inguinal and perirenal white adipose tissue (WAT) pads and the interscapular BAT depot were removed and weighed. Brains were removed from the skull, and the hypothalami were dissected. Plasma leptin levels were measured using a commercially available mouse leptin radioimmunoassay kit according to the manufacturer's protocol (Linco Research, St. Charles, MO). The Animal Ethics Committee of Utrecht University approved all described experiments.

TaqMan real-time PCR assays. Total RNA was extracted from the hypothalamus using TRIzol (Life Technologies, Paisley, UK), and residual genomic DNA was removed by on-column RNase-free DNase treatment (Qiagen, West Sussex, UK). Removal of the residual genomic DNA was tested by PCR amplification of a nontranscribed sequence. Reverse transcription of RNA in cDNA was performed using random hexamers and Moloney murine leukemia virus RT (Applied Biosystems, Foster City, CA). mRNA levels for MC3R and NPY Y_1 receptor (NPY Y_1R) in individual whole hypothalami were quantified by TaqMan real-time PCR. TaqMan PCR reactions for MC3R and NPY Y_1R were carried out on triplicate cDNA samples or genomic DNA standards in 384-well optical plates on an ABI Prism 7900 HT Sequence Detection system (Applied Biosystems). Predesigned gene expression assays were used for MC3R and NPY Y_1R genes (Mm 00434876-s1 and Mm 00650798-g1, respectively; Applied Biosystems). To normalize for differences in the expression data, mouse β-actin (ACTB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Applied Biosystems) were used as endogenous controls. Relative expression of the gene was determined by comparing its expression with that of endogenous control genes. Data evaluation was carried out using SDS 2.1 software. The comparative threshold cycle (C_T) method was used to determine the relative quantification of target genes; a fluorescence threshold is chosen, and the C_T values are calculated by determining the cycle number at which the fluorescence exceeds the C_T.

Statistical analysis. Data are expressed as means ± SE. Differences in body weight, food intake, motor activity, and body temperature were assessed by a general linear model repeated-measures procedure using a between-subject factor (strain) and within-subject factor (days). Relative body weight on *day 4* of food restriction, average body weight, food intake, and locomotor activity during baseline were analyzed by one-way ANOVA. When a significant difference ($P < 0.05$) between strains was observed, the analysis was followed by Bonferroni post hoc analysis. The WAT/BAT masses and plasma

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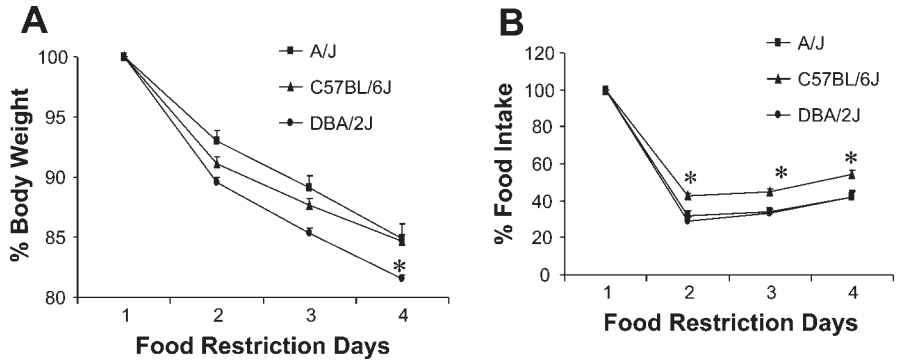

Fig. 1. Relative body weight (*A*) and food intake (*B*) during food restriction in 3 inbred strains. *A*: under baseline conditions, the body weight of DBA/2J and C57BL/6J mice was higher than that of A/J mice (see text). Because of substantial weight loss in DBA/2J mice, the relative body weight (i.e., %initial weight) on the last day of scheduled food access was significantly lower than both C57BL/6J and A/J mice ($P = 0.01$). *Group differences on *day 4* ($P < 0.05$). *B*: absolute food intake under baseline conditions was lower in C57BL/6J compared with DBA/2J and A/J mice (see text). However, relative food intake during the restriction period was lower in DBA/2J and A/J compared with C57BL/6J mice ($P = 0.0001$). *Group differences on each day of restriction ($P < 0.05$).

leptin levels were analyzed by two-way ANOVA with strain and experimental condition factors. Student's *t*-test was performed to determine the significance of the difference in gene expression between two experimental conditions in each strain. Data were analyzed using SPSS 11.5 for Windows.

RESULTS

Difference in body weight and food intake across strains. Under baseline conditions, the absolute body weight of the DBA/2J and C57BL6/6J mice (30.70 ± 0.79 and 32.01 ± 0.47 g, respectively) was significantly higher than that of the A/J mice [28.25 ± 0.57 g; $F(2,21) = 10.59$; $P = 0.001$]. During food restriction, DBA/2J mice showed substantial weight loss, and, on the last day of food restriction, the body weight of the DBA/2J mice as a percentage of baseline was significantly lower compared with both C57BL/6J and A/J mice [$F(2,21) = 5.24$; $P = 0.01$; Fig. 1*A*].

During the baseline period, food intake corrected to body weight was lower in C57BL/6J (0.13 ± 0.00 g/g body wt) compared with both the DBA/2J and A/J mice [0.16 ± 0.01 g/g body wt; $F(2,21) = 8.85$; $P = 0.002$]. However, under restricted food access conditions, food intake as a percentage of baseline was higher in C57BL/6J compared with DBA/2J and A/J mice, indicating that there is a larger relative decrease in food intake in these latter two strains [$F(2,21) = 17.615$; $P = 0.0001$; Fig. 1*B*]. The relatively larger decrease in these two strains was not the result of different amounts of available food, since during the food restriction period, all strains had ad libitum food access during the first 2 h of the dark phase.

Body temperature and locomotor activity during baseline and restricted food access. Under baseline conditions, average daily body temperature was similar in three strains (35.9 ± 0.08°C in A/J, 36.6 ± 0.13°C in C57BL/6J, and 36.7 ± 0.07°C in DBA/2J). However, under food restriction, body temperature relative to baseline was significantly lower in the C57BL/6J and DBA/2J compared with the A/J mice [$F(2,18) = 14.00$; $P = 0.0001$; Fig. 2*A*].

The total daily locomotor activity under baseline conditions was significantly lower in the A/J (377 ± 21.31 counts/day) compared with the C57BL/6J and DBA/2J mice [716 ± 51.26 and 854 ± 48.71 counts/day, respectively; $F(2,18) = 11.68$; $P = 0.001$]; i.e., locomotor activity is ~50% less in the A/J strain. The locomotor activity in C57BL/6J mice does not change over the entire food restriction period; in contrast, starting from *day 2*, the DBA/2J and A/J mice exhibited a significant increase in locomotor activity during restricted food access [$F(2,18) = 6.61$; $P = 0.007$; Fig. 2*B*].

Tissue weights and plasma leptin measurements. Figure 3 shows the WAT (*A*) and BAT (*B*) weights corrected for body weight in the three strains during baseline and food-restricted conditions. There was a decrease in WAT weight in all strains under restricted food conditions [experimental condition $F(1,43) = 39.70$; $P = 0.0001$]. In addition, there was an interaction effect between strain and experimental conditions [strain × experimental condition $F(2,43) = 6.94$; $P = 0.002$], since the decrease in WAT weight under food restriction varies significantly between the three strains (60.6% in A/J, 48.8% in C57BL/6J, and 72.0% in DBA/2J).

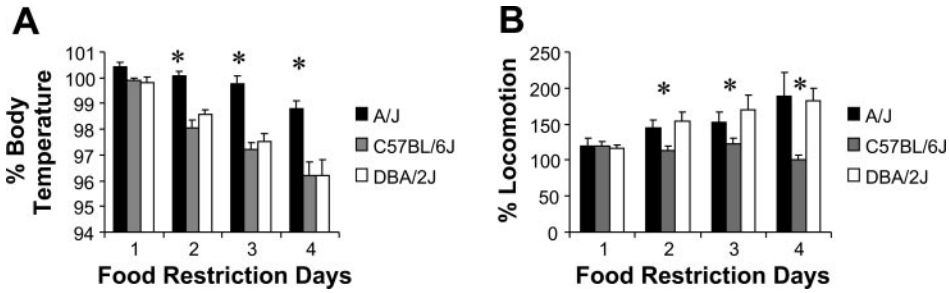

Fig. 2. Relative body temperature (*A*) and locomotor activity (*B*) during food restriction in 3 inbred strains. *A*: average daily body temperature was similar in the 3 strains under baseline conditions (see text). However, there was a significant decrease in the relative body temperature in both the C57BL/6J and DBA/2J strains compared with the A/J mice ($P = 0.0001$) during the food restriction period. The difference between strains was significant on *days 2*, *3*, and *4* (*$P < 0.05$). *B*: total daily locomotor activity under baseline conditions was significantly and much lower in A/J compared with C57BL/6J and DBA/2J mice (see text). From *day 2* of food restriction, an increase in locomotor activity was seen in DBA/2J and A/J mice; however, the C57BL/6J mice did not change their locomotor activity over the restriction period ($P = 0.007$). The difference between C57BL/6J and the other two strains was significant on *days 2*, *3*, and *4* (*$P < 0.05$).

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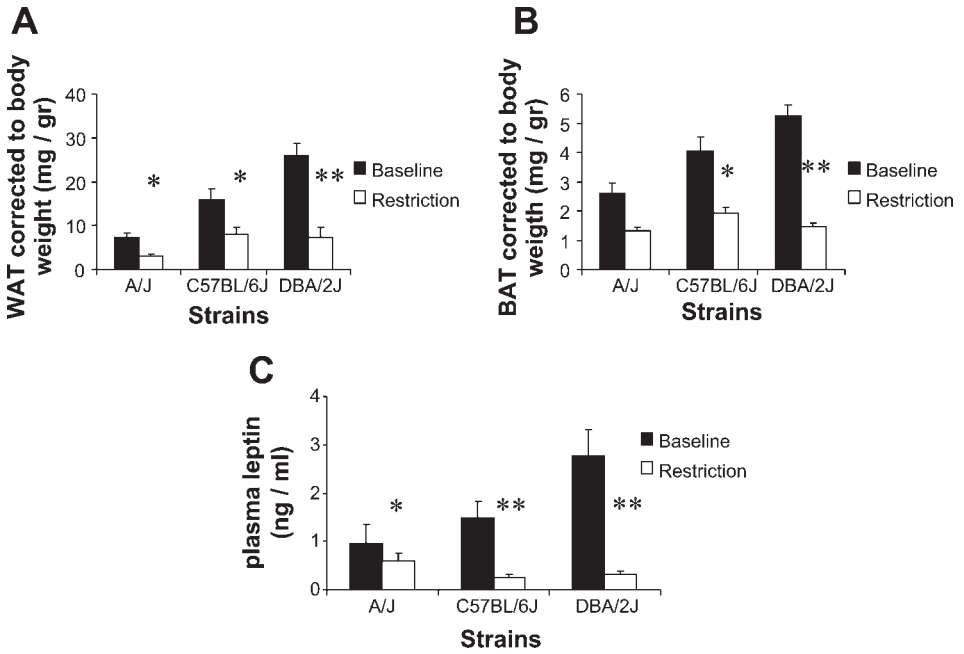

Fig. 3. White adipose tissue (WAT; *A*) and brown adipose tissue (BAT; *B*) weights and plasma leptin levels (*C*) under baseline and restriction conditions in 3 inbred strains. There was a decrease in both WAT and BAT weights in all strains (*$P = 0.0001$ for experiment); however, the decrease in DBA/2J is more substantial compared with the other two strains (**$P = 0.002$ for strain × experiment for WAT; **$P = 0.001$ for strain × experiment for BAT weight). Plasma leptin levels decreased in the 3 strains under scheduled food access (*$P = 0.0001$ for experiment); however, this decrease was more significant in DBA/2J and C57BL/6J mice compared with A/J (**$P = 0.014$ for strain × experiment).

There was a decrease in BAT weight in all strains under restricted food access [experimental condition $F(1,43) = 83.50$; $P = 0.0001$] and a combined effect of strain and experimental conditions [strain × experimental condition $F(2,43) = 8.04$; $P = 0.001$], with the decrease in BAT weight in the DBA/2J strain being significantly greater (72%) compared with A/J and C57BL/6J mice (48.7 and 52.5%, respectively).

Plasma leptin levels decreased in all strains during scheduled food access [experimental condition $F(1,43) = 23.14$; $P = 0.0001$]. As in the case of WAT and BAT weights, there was also a combined effect of strain and experimental condition [strain × experimental condition $F(2,43) = 4.68$; $P = 0.014$], since the decrease in the DBA/2J and C57BL/6J strains is greater (88.5 and 83.2%, respectively) than in the A/J mice (38.6%; Fig. 3*C*).

MC3R and NPY Y_1R expression in the hypothalamus. There was a significant decrease in the expression of the MC3R in the A/J and C57BL/6J mice during food restriction [relative to both of the control genes (β-actin, GAPDH)], whereas there was no change in the DBA/2J strain (Fig. 4, *left*, and Table 1). There was a significant increase in the relative expression of the NPY Y_1R in DBA/2J mice under food restriction; this was in contrast to the other two strains, which did not show a difference (Fig. 4, *right*, and Table 2). As can be seen from Fig 4, *right*, there was a slight increase in the relative expression of NPY Y_1R in C57BL/6J mice, but this was not significant (Table 2).

DISCUSSION

This study shows that three different inbred strains of mice exhibit distinct behavioral, physiological, and molecular differences in response to food restriction. Whereas C57BL/6J mice do not change their locomotor activity, the DBA/2J and A/J strains increase their activity during food restriction, and this increase is accompanied with a reduction in body temperature in both strains that is significantly larger in DBA/2J compared with A/J mice. C57BL/6J mice do not respond with increased physical activity and showed decreased thermogenesis. Changes in body temperature and locomotor activity are accompanied with changes in WAT/BAT masses, plasma leptin levels, and MC3R/NPY Y_1R expression in the hypothalamus, which will be discussed subsequently. Our data show that

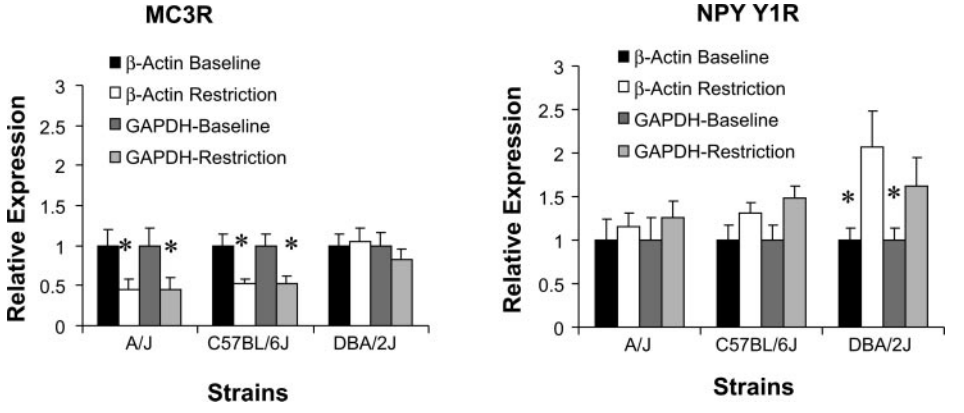

Fig. 4. Relative expression of melanocortin 3 receptor (MC3R) and neuropeptide Y Y_1 receptor (NPY Y_1R) under baseline and restriction conditions in 3 inbred strains. *The relative expression of MC3R is decreased in A/J and C57BL/6J strains under food restriction; on the other hand, there was an increase in the relative expression of NPY Y_1R in DBA/2J. Furthermore, these gene expression effects are consistent when tested against both endogenous controls [β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)].

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Table 1. *Relative expression of MC3R in 3 inbred strains under baseline and restriction conditions against 2 endogenous control genes*

	A/J		C57BL/6J		DBA/2J	
MC3R	β-Actin	GAPDH	β-Actin	GAPDH	β-Actin	GAPDH
Baseline	1 (0.85–1.2)	1 (0.84–1.2)	1 (0.88–1.14)	1 (0.89–1.13)	1 (0.87–1.15)	1 (0.87–1.15)
Restriction	0.45 (0.37–0.58)	0.45 (0.36–0.59)	0.52 (0.46–0.58)	0.53 (0.47–0.61)	1.05 (0.91–1.21)	0.83 (0.72–0.95)
P value (baseline/restriction)	0.0001	0.0001	0.004	0.002	0.885	0.557

Values are means ± SE. MC3R, melanocortin 3 receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Student's *t*-test was performed to determine the significance of the difference in gene expression between baseline and restriction conditions in each strain.

genetic background is an important parameter determining the animal's strategy in response to restricted feeding and that the observed strain differences have the potential to be used to identify molecular and physiological determinants of adaptive mechanisms in response to changes in food availability, e.g., through mapping studies.

During baseline conditions, the food intake is higher in DBA/2J and A/J strains than in the C57BL/6J but becomes lower than that of C57BL/6J during scheduled food access. As a consequence, the relative decrease in food intake in DBA/2J and A/J mice is larger. This could be because of a maladaptive behavior in response to restricted feeding or because of a lower stomach capacity for the food that can be ingested. However, irrespective of the cause underlying a larger decrease in food intake in DBA/2J and A/J strains, it is important to note that, from the second day of food restriction, DBA/2J and A/J mice increase their locomotor activity, whereas C57BL/6J mice either do not significantly change or slightly decrease their activity level. The effect of food restriction is significantly greater in DBA/2J and A/J mice because of the larger decrease in relative food intake during restricted food access. Therefore, DBA/2J and A/J mice may display behavior whereby the degree of restriction increases to the point where DBA/2J and A/J mice have a life-threatening food deprivation that is forcing them into energy-expensive behavioral hyperactivity. This suggests that behavioral hyperactivity during negative energy balance would be related to the degree of energy imbalance rather than to a different adaptation strategy to food restriction. If this is true, C57BL/6J mice might exhibit a similar increase in behavioral activity if subjected to the same level of food restriction as observed in DBA/2J and A/J mice. However, as can be seen from Fig. 1, on *day 4* of restricted feeding, when the relative body weight is similar in C57BL/6J and A/J strains, there is a 189% increase in locomotor activity in A/J mice but not in the C57BL/6J strain. Similarly, on *day 2* of scheduled feeding, when the relative body weight in C57BL/6J is only slightly higher than that of DBA/2J mice, there is a 154% increase in locomotor activity in DBA/2J mice but no change in the activity level of the C57BL/6J strain. Furthermore, although the decrease in plasma leptin levels is similar in C57BL/6J and DBA/2J strains, only the DBA/2J strain displayed increased behavioral activity during food restriction. In addition, the A/J strain displayed an increase in locomotor activity during food restriction similar to the DBA/2J strain despite the lack of a large decrease in plasma leptin level. There is a strain-dependent correlation between the WAT depot mass and plasma leptin level, with a lack of correlation in the A/J strain ($r = 0.257$; $P = 0.33$) and a strong positive correlation in DBA/2J and C57BL/6J mice ($r = 0.823$, $P = 0.0001$ and $r = 0.933$, $P = 0.0001$, respectively). These strain-dependent correlations are also reflected in studies of diet-induced obesity. For example, A/J, DBA/2J, and C57BL/6J strains are commonly used in obesity and diabetes research as obesity-resistant (A/J, no correlation of WAT with leptin) and obesity-prone (C57BL/6J and DBA/2J, strong positive correlation of WAT with leptin) strains (1, 8, 18, 52, 53). It is known that the levels of circulating leptin are important indicators of adiposity, transferring information to the hypothalamus regarding the amount of energy stored in adipose tissue that will eventually lead to the suppression of appetite and increased energy expenditure (17, 40). Although low plasma leptin levels are involved in signaling negative energy status, we have not observed a direct relationship between hypoleptinemia and the development of increased locomotor activity in male mice under the conditions of this study. Taken together, these data suggest that the hyperactivity in DBA/2J and A/J strains is more likely to be because of the adoption of a different strategy in response to the scheduled feeding paradigm rather than the degree of negative energy status.

Decreasing physical activity in response to food deprivation contributes to energy saving (54). When the available food is reduced, animals may reduce their locomotor activity to decrease energy expenditure, or alternatively the restriction may induce energy-expensive behavioral hyperactivity, either for

Table 2. *Relative expression of NPY Y_1R in 3 inbred strains under baseline and restriction conditions against 2 endogenous control genes*

	A/J		C57BL/6J		DBA/2J	
NPY Y_1R	β-Actin	GAPDH	β-Actin	GAPDH	β-Actin	GAPDH
Baseline	1 (0.8–1.24)	1 (0.8–1.25)	1 (0.84–1.17)	1 (0.85–1.17)	1 (0.88–1.14)	1 (0.87–1.14)
Restriction	1.15 (1.01–1.31)	1.25 (1.09–1.44)	1.31 (1.21–1.42)	1.48 (1.37–1.61)	2.07 (1.76–2.48)	1.62 (1.37–1.95)
P value (baseline/restriction)	0.781	0.403	0.172	0.082	0.006	0.012

Values are means ± SE. NPY Y_1R, neuropeptide Y Y_1 receptor. Student's *t*-test was performed to determine the significance of the difference in gene expression between baseline and restriction conditions in each strain.

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food foraging or maintaining body temperature. Many animals increase their physical activity during the later phases of food deprivation, a response that has been proposed to reflect an immediate need for food (54). During prolonged food deprivation in rats, locomotor activity at the end phase of fasting (which is characterized by the depletion of adipose tissue) was 10 times higher than under baseline conditions (34, 54). Furthermore, in a study where the golden spiny mouse *Acomys russatus* was exposed to prolonged food deprivation, an 83% decrease in metabolic activity was observed, and this was accompanied by a decrease of 3.5°C in temperature. In parallel, the animals displayed an increase in locomotor activity in the active period of their diurnal cycle (12). We observed increased locomotor activity in DBA/2J and A/J strains from *day 2* of food restriction, in contrast to the C57BL/6J strain.

In general, changes in locomotor activity levels during food restriction are coupled to core body temperature (12, 23, 26, 38, 58). During food restriction, A/J mice have increased motor activity levels, which are accompanied with a reduction in body temperature. On the other hand, C57BL/6J mice do not respond with increased motor activity and indeed display a larger decrease in their core body temperature compared with A/J mice. Most surprisingly, the DBA/2J mice increase their motor activity, as seen in A/J mice, but also exhibit a significantly larger drop in body temperature compared with the A/J strain; this could be because of a lack of coupling between physical activity and temperature regulation in DBA/2J mice. In addition to the lack of behavioral thermoregulation, among the three inbred strains, the DBA/2J mice have the most substantial decrease in WAT/BAT mass and plasma leptin levels at the end of food restriction. Leptin increases body core temperature (40), oxygen consumption, and UCP mRNA levels in BAT (48), which supports the observation that it increases energy expenditure through increased thermogenesis in BAT and WAT (9). Therefore, the failure to maintain body temperature despite increased locomotor activity in the DBA/2J strain can be explained from the observed large decrease in adipose tissue and plasma leptin levels, given the main role of BAT in heat generation (2, 49), the function of WAT in BAT thermogenesis (22), and leptin's effect on energy expenditure and thermogenesis (9, 40, 48).

NPY is involved in the regulation of food intake and energy homeostasis (3, 56, 59). NPY suppresses the sympathetic activity that activates thermogenesis in BAT (11), and direct injection of NPY in the paraventricular nucleus of the hypothalamus reduces UCP mRNA levels in BAT (4, 11). Six NPY receptors have been identified (5), and, of these, Y_1 and Y_5 receptors have been implicated in the control of food intake and energy utilization (29, 39). NPY Y_1 receptor-deficient mice have upregulated UCP1 expression in BAT, which might be the result of the lack of the inhibitory effects of NPY through Y_1 receptor on BAT metabolic activity (35). In our study, DBA/2J mice (but not C57BL/6J and A/J) have an upregulated NPY Y_1 receptor under scheduled feeding. The large decrease in body temperature in DBA/2J mice during food restriction might be the result of an increased antithermogenic effect of NPY through the Y_1 receptor as a consequence of its increased expression. Because leptin inhibits hypothalamic NPY synthesis (56), low leptin levels as seen in the DBA/2J strain would lead to an activation of NPY-synthesizing neurons, which in turn might lead to upregulation of NPY receptors. Although the decrease in plasma leptin level is similar in both DBA/2J and C57BL/6J strains, upregulation of NPY Y_1 receptor is only observed in the DBA/2J strain. One explanation could be that, in the C57BL/6J strain, other NPY receptors (e.g., NPY Y_5) mediate its antithermogenic effect in BAT.

The melanocortin system is involved in regulating food intake and energy expenditure (3, 56, 59), and both MC3R and MC4R knockout mice exhibit abnormalities in energy expenditure that contribute to their obese phenotypes (6, 51). Intracerebroventricular administration of an MC3/4R agonist increases both sympathetic nerve activity in BAT and body temperature (25, 36). We observed a downregulation of MC3R in A/J and C57BL/6J (but not DBA/2J) mice during restricted feeding. Downregulation of the MC3R and the lack of behavioral thermoregulation could explain the decrease in temperature in C57BL/6J mice during food restriction. In A/J mice, the reduced heat production possibly due to the downregulation in MC3R seems to be compensated by behavioral thermoregulation.

Changes in gene expression are known to be affected by energy intake and differences in macronutrient and micronutrient composition of the diet (19, 21, 31, 32, 42). Therefore, the differential expression of the NPY Y_1 and MC3R genes in the restricted mice could be because of the macronutrient/micronutrient deficiency rather than the restricted food intake. However, it should be noted that the three inbred strains used in this study were fed the same diet; thus, the nutrient composition of the diet was the same for each inbred strain, suggesting that the differential gene expression profile across the three strains was due to differences in food intake during restricted access. Further studies using diets with different macronutrient/micronutrient contents would allow assessment of the effect of dietary composition on the expression profile of the genes coding for NPY Y_1 and MC3Rs in the hypothalamus.

The scheduled feeding paradigm used in this study results in different behavioral, physiological, and molecular responses in three inbred strains. In the A/J strain, there is an increase in physical activity during the period of food restriction, and this is associated with a reduction in body temperature to a lesser extent compared with the DBA/2J and C57BL/6J mice. Although downregulation of MC3R will decrease thermogenesis, the A/J mice do not display a large decease in body temperature over the restriction period, possibly by behavioral hyperactivity. In C57BL/6J mice, the expression pattern for NPY Y_1 and MC3R is similar to that in A/J mice, but they display a larger decrease in their body temperature during restricted feeding compared with A/J mice. This may be because of the observed lack of increase in locomotor activity. In the DBA/2J strain, although the thermogenic MC3R is not downregulated during the restriction period, there is an upregulation of the antithermogenic NPY Y_1 receptor, which will impair heat production. The larger decrease in BAT and WAT adipose tissue masses during food restriction compared with A/J and C57BL/6J strains leads to decreased temperature in DBA/2J mice because of their main involvement in the heat production. Furthermore, despite increased physical activity, this behavioral thermoregulation in the DBA/2J strain is not associated with temperature maintenance. Consequently, as a result of a deregulation in behavioral thermoregulation and impaired heat

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production, DBA/2J mice display a large decrease in body temperature during food restriction.

Mouse CSS and RIS have been used to map a wide range of quantitative trait loci (QTLs; see Refs. 37, 50, and 57). Use of CSS and RIS in QTL mapping studies increases the power to detect and localize individual QTLs on chromosomal regions by reducing the phenotypic noise resulting from simultaneous segregation of multiple QTLs in large crosses performed in conventional QTL studies. In a panel of CSSs, the genome is partitioned into a collection of single chromosome substitutions on an isogenic background, whereas, in a panel of RIS of mice, the genome of the progenitor inbred strains is fragmented in a random and overlapped fashion. In both cases, the contribution of environmental factors and technical error to genetic variance seen across the progeny can be minimized, which consequently maximizes the heritability for QTL mapping studies (37, 50, 57). We have shown that three different inbred mice strains displayed behavioral, physiological, and molecular variations in their response to restricted feeding. The molecules underlying the development of different coping strategies to a food restriction paradigm could be mapped by phenotypically and genotypically testing individual strains forming an RIS and CSS panel, which in turn will lead to a better understanding of factors involved in the complex regulation of food intake and energy metabolism.

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