Original Article

Neuropeptide Y Deficiency Attenuates Responses to Fasting and High-Fat Diet in Obesity-Prone Mice

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Neuropeptide Y (NPY) stimulates feeding and weight gain, but deletion of the *NPY* gene does not affect food intake and body weight in mice bred on a mixed genetic background. We reasoned that the orexigenic action of NPY would be evident in C57Bl/6J mice susceptible to obesity. NPY deficiency has no significant effect in mice fed a normal rodent diet. However, energy expenditure is elevated during fasting, and hyperphagia and weight gain are blunted during refeeding. Expression of agouti-related peptide (AGRP) in the hypothalamus is increased in NPY knockout (NPYko) than wild-type mice, but unlike wild type there is no further increase in AGRP when NPYko mice are fasted. Moreover, NPYko mice have higher oxygen consumption and uncoupling protein-1 expression in brown adipose tissue during fasting. The failure of an increase in orexigenic peptides and higher thermogenesis may contribute to attenuation of weight gain when NPYko mice are refed. C57Bl/6J mice lacking NPY are also less susceptible to diet-induced obesity (DIO) as a result of reduced feeding and increased energy expenditure. The resistance to DIO in NPYko mice is associated with a reduction in nocturnal feeding and increased expression of anorexigenic hypothalamic peptides. Insulin, leptin, and triglyceride levels increase with adiposity in both wild-type and NPYko mice. *Diabetes* 55:3091–3098, 2006

Energy balance involves a complex interaction between hormones, such as leptin, insulin, and glucocorticoids, and key neurons in the hypothalamus, brainstem, and other areas of the central nervous system (1). Neuropeptide Y (NPY) is expressed in the hypothalamus, stimulates food intake, decreases energy expenditure, and increases body weight when administered in the brain (1). Consistent with its role as an orexigenic peptide, hypothalamic NPY expression is increased during fasting and hypoglycemia and suppressed by feeding, leptin, and insulin (1). Despite the strong pharmacological evidence supporting its role in energy balance, genetic disruption of NPY did not affect feeding or body weight in mice bred on a mixed 129J-C57Bl/6J background (2). Paradoxically, deletion of NPY or the Y1 and Y5 receptors that mediate effects of NPY on energy balance resulted in mild obesity (3–6). Nonetheless, loss of NPY attenuated hyperphagia and obesity in $Lep^{ob/ob}$ mice (7). Others have also shown that NPY deficiency decreases hyperphagia after fasting and hypoglycemia (8–10). Furthermore, deletion of NPY Y2/Y4 receptors prevents diet-induced obesity (DIO) (11).

We reasoned that the controversies surrounding the actions of NPY in these genetic models are partly due to the use of the 129 mouse strain, which is resistant to obesity (12). The seemingly normal phenotype of NPY-deficient mice could also result from compensatory developmental changes. For example, ablation of hypothalamic neurons expressing NPY/AGRP did not alter feeding in neonatal mice, whereas ablation in adults caused starvation (13,14). AGRP is coexpressed with NPY in the arcuate nucleus, and both peptides stimulate feeding and weight gain (1,15). It is possible that AGRP is increased when NPY is lacking, thus maintaining the ability to feed. Alternatively, anorexigenic peptides, e.g., proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), may be decreased in NPY-deficient mice, thereby preventing weight loss. We hypothesized that the importance of NPY in energy homeostasis would be evident in C57Bl/6J mice prone to obesity (12,14).

RESEARCH DESIGN AND METHODS

The studies were conducted in accordance with guidelines and regulations of the University of Pennsylvania Animal Care and Use Committee. Breeder $Npy^{+/-}$ mice on a mixed 129SvEv-C57Bl/6J background were provided by Richard Palmiter (University of Washington, Seattle, WA) and were backcrossed for 10 generations onto the C57BL/6J background (The Jackson Laboratories, Bar Harbor, ME). Heterozygotes were then bred to each other to generate NPYko mice and wild-type controls (2,3). The genotypes were confirmed by PCR as previously described (3). The mice were weaned at 3 weeks, housed five per cage at 22°C with 12 h light-dark cycles (lights on at 0600), and fed a rodent diet containing 4.5% fat, 49.9% carbohydrate, 23.4% protein, 4 kcal/g (Labdiet no. 5001; Labdiet, Richmond, IN) (16).

Fasting and refeeding studies. Mice aged 10 weeks were housed singly in calorimeter cages and habituated for 3 days. Body weight was measured, a preweighed amount of food was introduced at 0900, and oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) were measured at 15-min intervals using the following parameters: air flow 0.5 l, sample 0.4 l, settle

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AGRP, agouti-related peptide; BAT, brown adipose tissue; CART, cocaine- and amphetamine-regulated transcript; CRH, corticotropin-releasing hormone; DIO, diet-induced obesity; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus; UCP, uncoupling protein.

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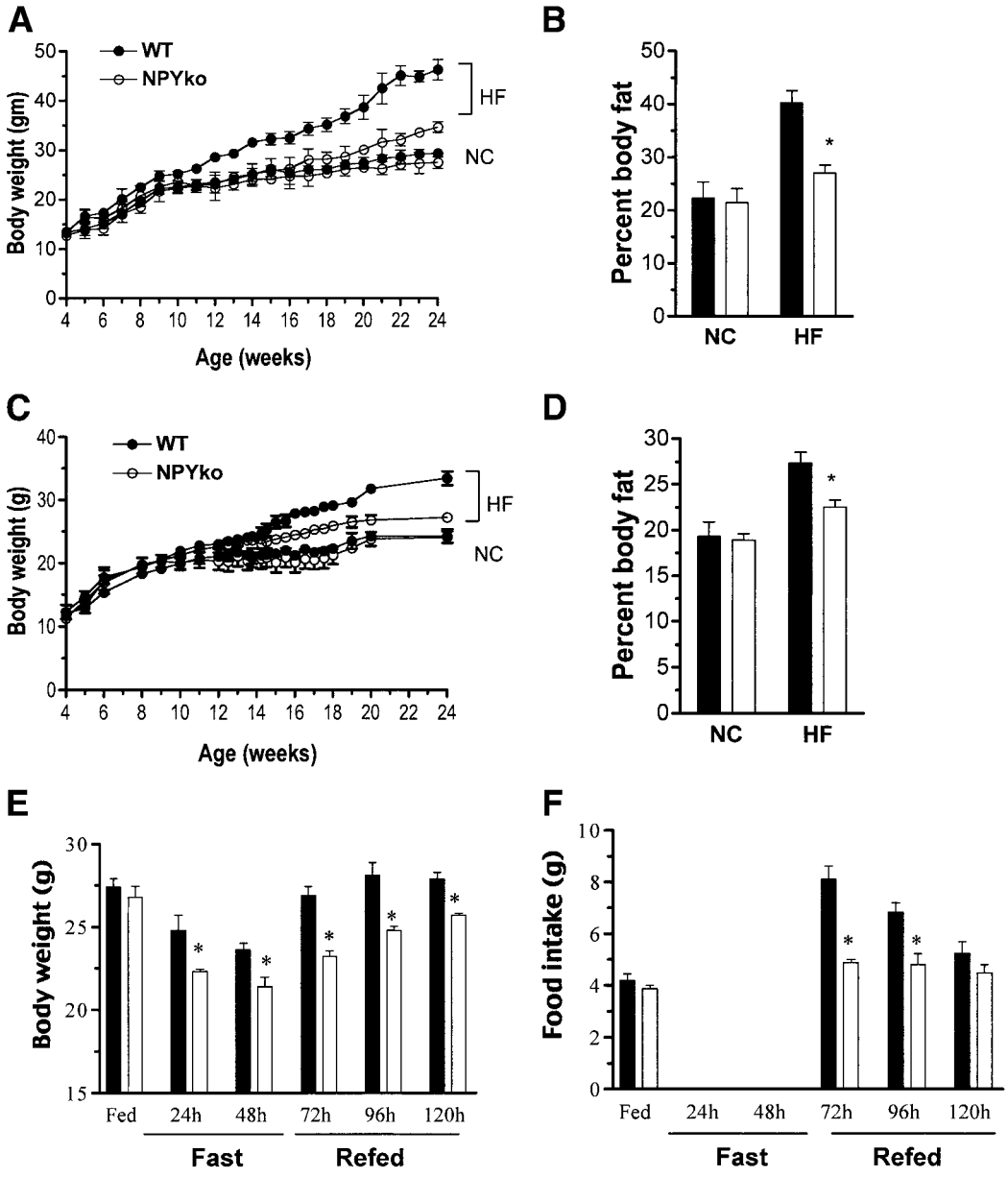

FIG. 1. Body weight and fat in male (*A* and *B*) and female (*C* and *D*) wild-type (WT; ■) and NPYko (□) mice fed normal chow (NC) or high-fat (HF) diets. Effects of fasting and refeeding on weight (*E*) and food intake (*F*). Data are means ± SE; $n = 6–8$. *$P < 0.001$ vs. wild type.

time 120 s, and measuring time 60 s (Oxymax EqualFlow System; Columbus Instruments, Columbus, OH) (16,17). The mice were fed ad libitum for 48 h or deprived of food for 48 h or refed for 48 h. Indirect calorimetry was performed throughout, except when food was removed or replaced. Locomotor activity was assessed simultaneously using photobeams (Optovarimex; Columbus Instruments). NPYko and wild-type mice fed, fasted, or refed as described were killed; blood was obtained via cardiac puncture, and brown adipose tissue (BAT) and hypothalami were rapidly excised, frozen in liquid nitrogen, and stored at −80°C.

Cold tolerance. Rectal temperature was measured at room temperature (time 0) in 10-week-old wild-type and NPYko male mice using a thermistor (YSI Model 4600) (16), after which the mice were housed at 4°C for 6 h.

In situ hybridization. NPYko and wild-type mice were anesthetized with sodium pentobarbital at 0900–1100 and perfused transcardially with diethyl pyrocarbonate–treated PBS followed by 10% neutral buffered formalin. The brains were excised, postfixed overnight, and cryoprotected in sucrose. NPY, AGRP, POMC, melanin-concentrating hormone (MCH), and corticotropin-releasing hormone (CRH) mRNA expression were detected on coronal sections (20 μm) using specific riboprobes as described (18,19).

DIO. NPYko and wild-type mice were fed a normal chow diet (4.5% fat, 49.9% carbohydrate, 23.4% protein, 4 kcal/g; Labdiet no. 5001; Labdiet) or a high-fat diet (45% fat, 35% carbohydrate, 20% protein, 4.7 kcal/g; Research Diets no. D12451; Research Diets, New Brunswick, NJ) from 4 weeks of age (16). Body weight was measured weekly. Food intake and body composition (dual-emission X-ray absorptiometry) and energy expenditure were measured at 4, 8, and 16 weeks (16,20). Feeding frequency (beam breaks) was assessed using the Vitalview System (Minimitter, Bend, OR). Diets were supplied in feeders equipped with photobeams, and feeding activity was analyzed using Actiview software (www.minimitter.com/Products/VitalView/index.html). The mice were killed at 0900–1200 the following day, blood was drawn via cardiac puncture, and hypothalami were harvested.

Tissue chemistry. Serum glucose, triglyceride, cholesterol, and nonesterified fatty acid levels were measured using colorimetric assays (Stanbio, Boerne, TX; Wako Chemicals, Richmond, VA) (16,17,21). Insulin and leptin were measured by enzyme-linked immunosorbent assay (CrystalChem, Chicago, IL). Corticosterone and thyroxine were measured by radioimmunoassay (16,17,21). Expression of uncoupling protein (UCP)-1 mRNA level in BAT was measured by Northern blot (17). Hypothalamic neuropeptide mRNA levels were measured using real-time PCR and normalized to ribosomal phosphoprotein (16,17).

Statistics. Effects of NPY deficiency on various parameters were analyzed by ANOVA and Fisher's protected least significant difference (PSLD) test. Regression analysis was performed on continuous variables. A P value <0.05 was considered significant.

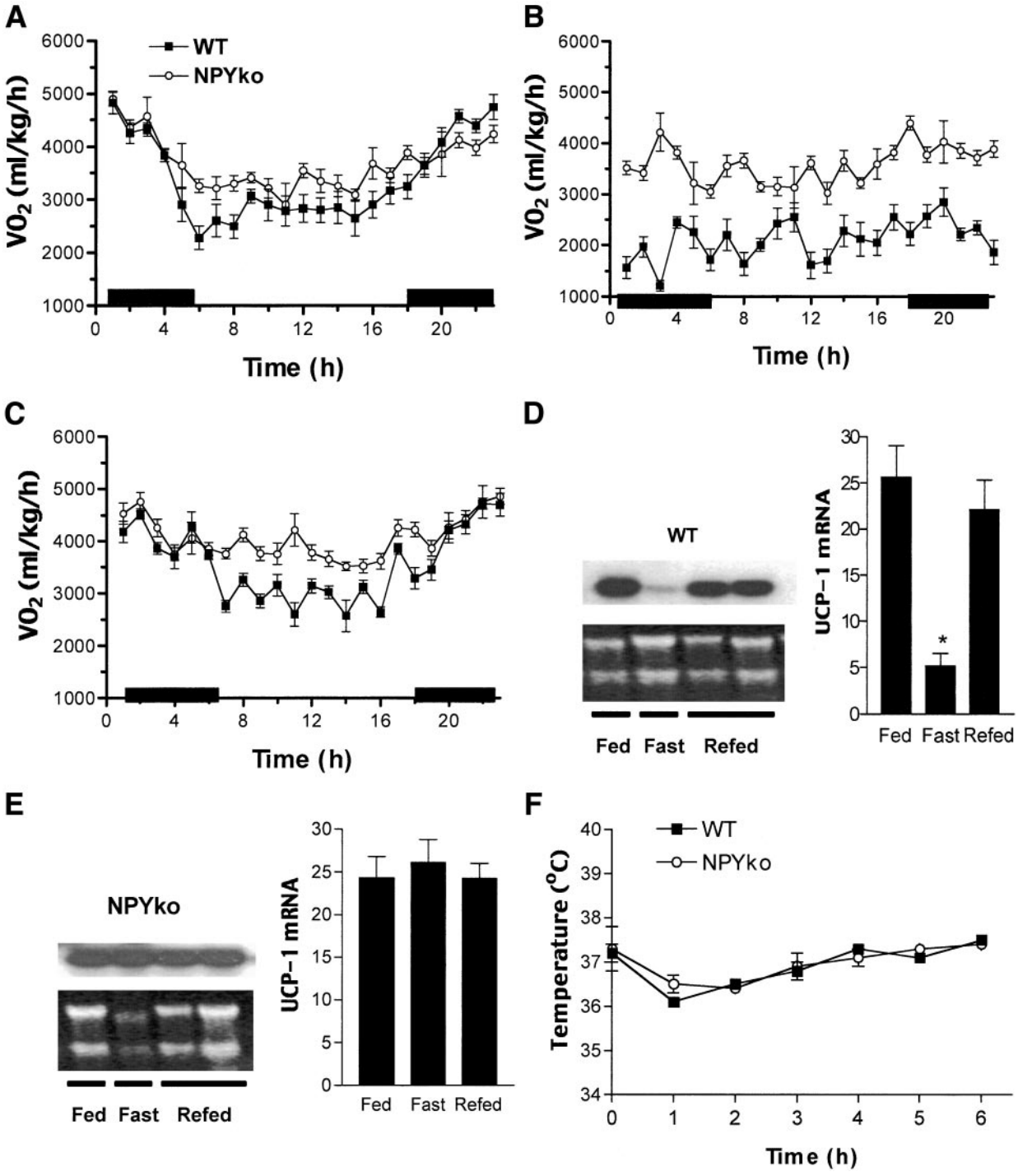

FIG. 2. Oxygen consumption (VO_2) in fed (*A*), fasted (*B*), and refed (*C*) male wild-type (WT) and NPYko mice. Light and dark periods are shown. Northern blot of BAT UCP-1 in wild-type (*D*) and NPYko (*E*) mice. *F*: Rectal temperature in wild-type and NPYko mice kept at 4°C. Data are means ± SE; $n = 6$. *$P < 0.0001$ vs. fed.

RESULTS

NPY deficiency disrupts metabolic responses to fasting. NPYko and wild-type littermates were born at the expected Mendelian frequencies (2,3). We did not observe spontaneous seizures in C57Bl/6J-NPYko mice in contrast to the original NPYko on the 129/C57Bl/6J strain (2). On normal chow diet, male and female NPYko grew at a normal rate, and the fat content was similar to wild type (Fig. 1*A*–*D*). We subjected wild-type and NPYko mice to fasting. Body weight decreased by 15% in wild-type mice after fasting and was associated with hyperphagia and rapid recovery of weight during refeeding (Fig. 1*E* and *F*). NPYko mice lost more weight than wild-type mice during fasting and exhibited blunting of hyperphagia and weight recovery during refeeding (Fig. 1*E* and *F*). Diurnal oxygen consumption (Vo_2) was lower than nocturnal in both wild-type and NPYko mice (Fig. 2*A*). Vo_2 fell significantly by 40–50% ($P < 0.0001$) in fasted wild-type mice and was restored after refeeding (Fig. 2*A*–*C*). The fall in Vo_2 in wild-type mice corresponded to a drastic reduction in BAT UCP-1 expression (Fig. 2*D*).

TABLE 1
Effect of NPY deficiency on chemistry in fed and fasted mice (n = 6–8)

	Wild type			NPYko		
	Fed	Fast	Refed*	Fed	Fast†	Refed*
Glucose (mg/dl)	128 ± 7.4	63 ± 3.7†	134 ± 6.5	109 ± 2.9	57 ± 3.8	126 ± 4.1
Triglyceride (mg/dl)	97 ± 3.5	74 ± 5.3	113 ± 2.8	88 ± 2.5	61 ± 1.3	83 ± 0.6
Insulin (ng/ml)	0.98 ± 0.1	0.41 ± 0.08†	1.4 ± 0.06	0.93 ± 0.02	0.37 ± 0.1	0.88 ± 0.07
Leptin (ng/ml)	4.31 ± 0.2	2.63 ± 0.1†	3.87 ± 0.01	3.68 ± 0.05	1.97 ± 0.1	3.56 ± 0.06
Thyroxine (mg/dl)	3.45 ± 0.01	1.48 ± 0.03†	3.27 ± 0.1	3.81 ± 0.1	1.87 ± 0.05	3.62 ± 0.04
Corticosterone (ng/ml)	78 ± 9.3	214 ± 36†	101 ± 7.2	84 ± 5.2	239 ± 12.5	113 ± 4.7

Data are means ± SE. Ten-week-old male wild-type and NPYko mice were fed ad libitum and were fasted for 48 h or fasted for 48 h and then refed for 48 h. They were euthanized at 0900–1200. *$P < 0.05$ compared with fasted mice; †$P < 0.05$ compared with fed mice.

In contrast to wild-type mice, V_{O_2} remained elevated in fasted and refed NPYko mice (Fig. 2*A–C*). Moreover, BAT UCP-1 expression did not fall in fasted NPYko mice (Fig. 2*E*). In contrast, wild-type and NPYko mice both maintained body temperature during cold exposure (Fig. 2*F*). NPY deficiency did not affect locomotor activity during fasting or refeeding (data not shown). As was the case in wild-type mice, glucose, insulin, leptin, thyroxine, and triglyceride levels fell and corticosterone increased in fasted NPYko mice (Table 1). These changes were reversed after refeeding (Table 1).

We determined whether NPY deficiency would affect the

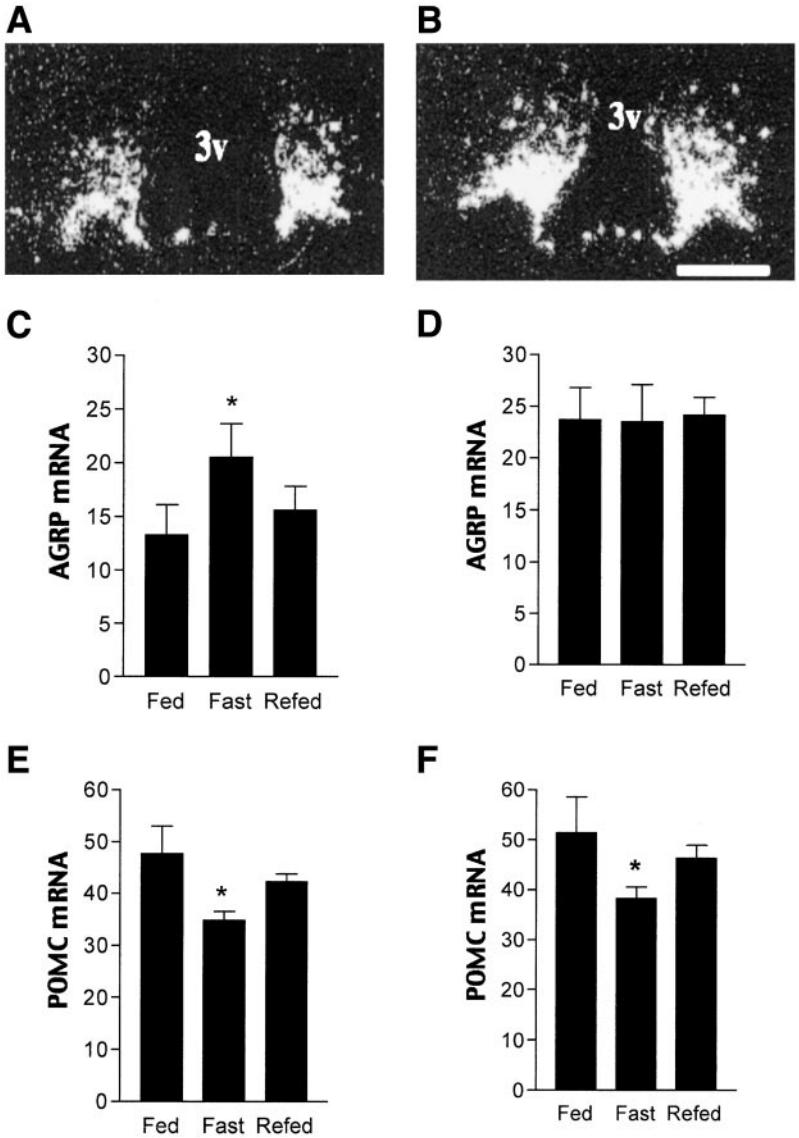

FIG. 3. Dark field photomicrographs showing AGRP mRNA expression in wild-type (*A*) and NPYko (*B*) mice. Scale bar = 150 μm. 3v, third ventricle. AGRP levels in wild-type (*C*) and NPYko (*D*) mice. POMC levels in wild-type (*E*) and NPYko (*F*) mice. Data are means ± SE; $n = 5$. *$P < 0.01$ vs. fed.

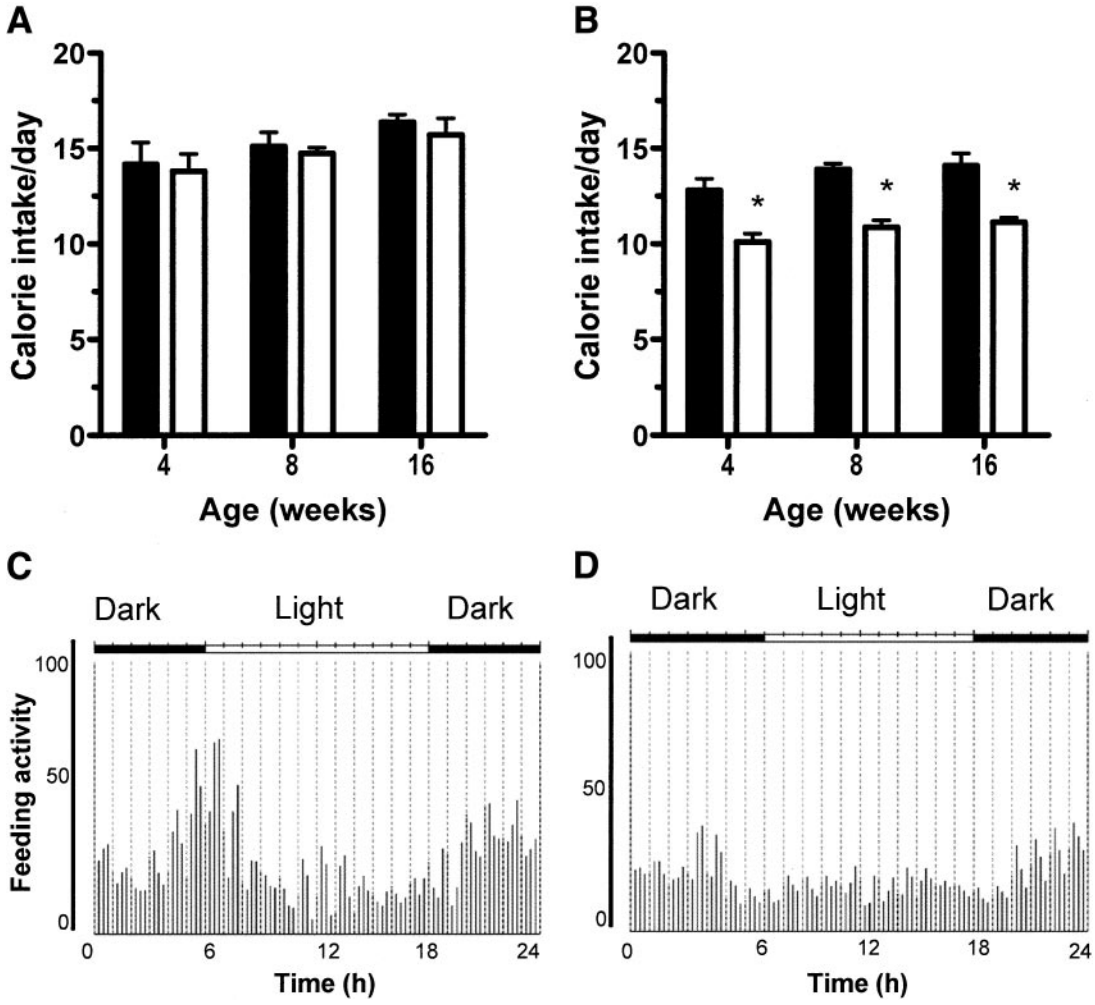

FIG. 4. Daily intake of normal chow (*A*) and high-fat (*B*) diets in wild-type (WT; ■) and NPYko (□) mice. Data are means ± SE; $n = 8$. *$P < 0.001$ vs. wild type. *C* and *D*: Feeding activities in 8-week-old high-fat wild-type (*C*) and high-fat NPYko (*D*) mice.

levels of hypothalamic neuropeptides implicated in energy homeostasis (1). Expression of AGRP was confined to the arcuate nucleus in both wild-type and NPYko mice (Fig. 3*A* and *B*). However, AGRP mRNA levels were two times higher in NPYko than wild-type mice (Fig. 3*A–D*). AGRP was increased by fasting and suppressed by refeeding in wild-type mice (Fig. 3*C*). In contrast, there were no obvious changes in AGRP mRNA levels during fasting or refeeding in NPYko mice (Fig. 3*D*). The distributions of POMC, CRH, and MCH were not altered by NPY deficiency (data not shown). POMC expression fell in response to fasting in both wild-type and NPYko mice and increased after refeeding (Fig. 3*E* and *F*). MCH increased and CART decreased in fasted wild-type and NPYko mice, and these changes were reversed by refeeding (data not shown).

NPY deficiency attenuates DIO in C57Bl/6J mice. Male and female NPYko mice showed significant reductions of body weight and fat content on a high-fat diet (Fig. 1*A–D*). Body weight was significantly lower in high-fat male NPYko mice by 12 weeks and female NPYko mice by 16 weeks compared with high-fat wild-type mice ($P < 0.001$). Body fat was 25% lower in male NPYko mice and 15% lower in female NPYko mice (Fig. 1*B* and *D*). Daily consumption of normal chow diet was similar in wild-type and NPYko mice (Fig. 4*A*). In contrast, high-fat intake was markedly reduced in NPYko mice (Fig. 4*B*). Moreover, feeding frequency was attenuated at night in high-fat NPYko mice (Fig. 4*C* and *D*). The light-to-dark feeding ratio was 0.67 ± 0.1 in high-fat NPYko mice compared with 0.28 ± 0.06 in high-fat wild-type mice ($P < 0.0001$). Vo_2 was also higher in high-fat NPYko mice (3,778 ± 66 ml · kg^{-1} · h^{-1}) than high-fat wild-type mice (3,415 ± 48 ml · kg^{-1} · h^{-1}) ($P < 0.05$).

Hypothalamic AGRP mRNA levels were higher in NPYko than wild-type mice on normal chow and high-fat diets ($P < 0.0001$) (Fig. 5*A*). POMC and CRH levels were similar in normal chow wild-type and NPYko mice but increased markedly when NPYko mice were switched to a high-fat diet (Fig. 5*B* and *C*) ($P < 0.0001$). Figures 5*D–F* show the relationships between body fat and hypothalamic neuropeptide expression. AGRP was inversely related to fat in both genotypes, but this was not significant (wild type $r = -0.149$, $P = 0.595$; NPYko $r = -0.269$, $P = 0.351$) (Fig. 5*D*). POMC decreased with adiposity in wild-type ($r = -0.536$, $P = 0.039$) but increased in NPYko ($r = 0.559$, $P = 0.037$) mice (Fig. 5*E*). CRH did not change with adiposity in wild-type ($r = 0.115$, $P = 0.682$) but increased in NPYko ($r = 0.552$, $P = 0.04$) mice (Fig. 5*F*). Expression of MC4 receptor, CART, and MCH did not change with adiposity in wild-type or NPYko mice (data not shown).

Serum levels of glucose (wild type $r = 0.744$, $P = 0.0014$; NPYko $r = 0.712$, $P = 0.004$), insulin (wild type $r = 0.865$,

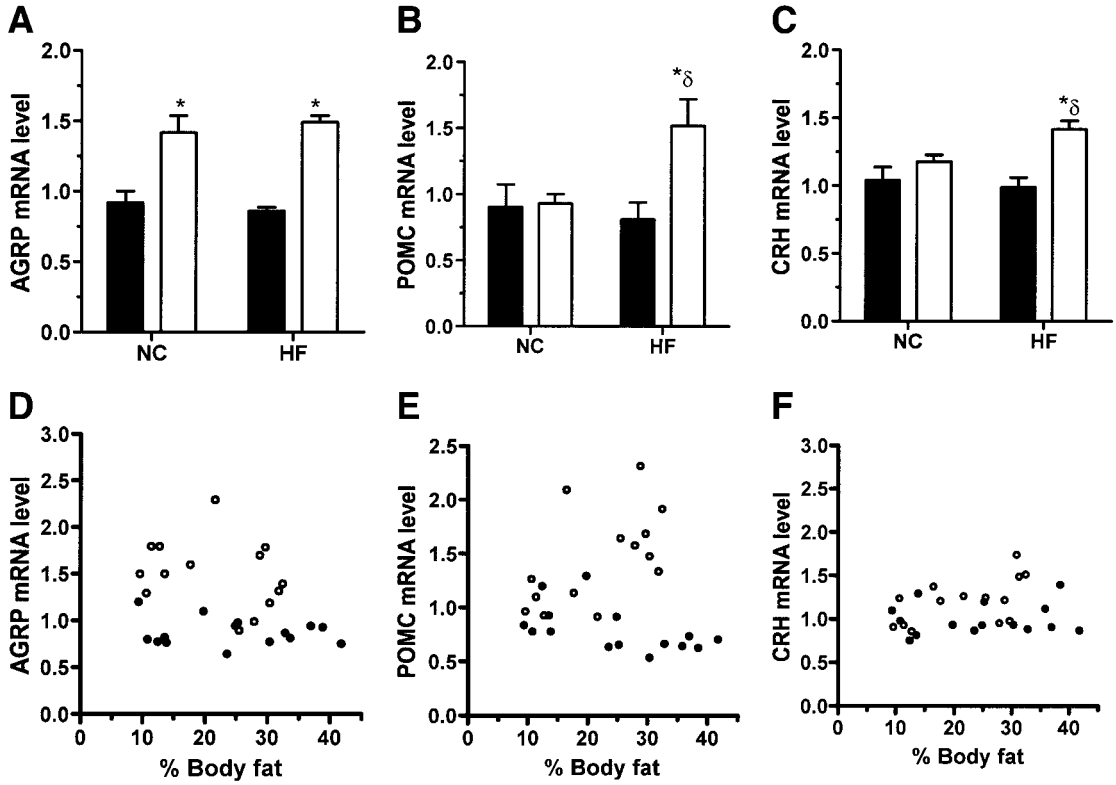

FIG. 5. *A–C*: Hypothalamic neuropeptide expression in 8-week-old normal chow (NC) and high-fat (HF) wild-type (■) and NPYko (□) mice. Data are means ± SE; $n = 5$. *$P < 0.0001$ vs. normal chow wild type; δ$P < 0.0001$ vs. high-fat wild type. Relationships between body fat and hypothalamic neuropeptide expression levels in wild-type (●) and NPYko (○) mice aged 4–16 weeks. *D*: AGRP (wild type $r = -0.149$, $P = 0.595$; NPYko $r = -0.269$, $P = 0.351$). *E*: POMC (wild type $r = -0.536$, $P = 0.039$; NPYko $r = 0.559$, $P = 0.037$). *F*: CRH (wild type $r = 0.115$, $P = 0.682$; NPYko $r = 0.552$, $P = 0.04$).

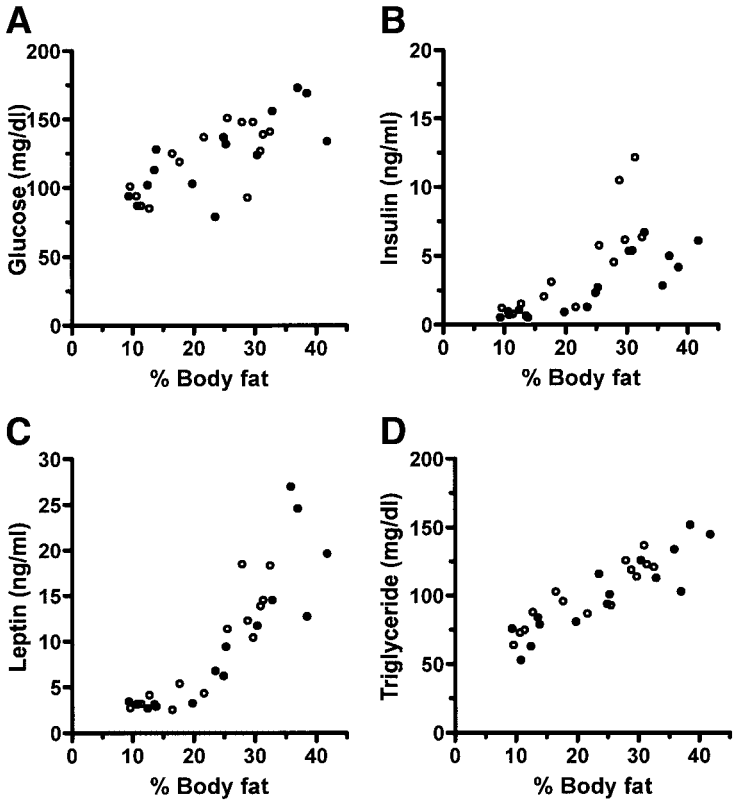

FIG. 6. Relationship between serum chemistry and body fat in DIO wild-type (●) and NPYko (○) mice. *A*: Glucose (wild type $r = 0.744$, $P = 0.0014$; NPYko $r = 0.712$, $P = 0.004$). *B*: Insulin (wild type $r = 0.865$, $P < 0.0001$; NPYko $r = 0.813$, $P = 0.0004$). *C*: Leptin (wild type $r = 0.866$, $P < 0.0001$; NPYko $r = 0.8977$, $P < 0.0001$). *D*: Triglyceride (wild type $r = 0.902$, $P < 0.0001$; NPYko $r = 0.902$, $P < 0.0001$).

$P < 0.0001$; NPYko $r = 0.813$, $P = 0.0004$), leptin (wild type $r = 0.866$, $P < 0.0001$; NPYko $r = 0.8977$, $P < 0.0001$), and triglyceride (wild type $r = 0.902$, $P < 0.0001$; NPYko $r = 0.902$, $P < 0.0001$) levels increased in relation to body fat (Fig. 6*A–D*), whereas thyroxine and corticosterone were not affected by body fat (data not shown).

DISCUSSION

Here, we report that ablation of NPY attenuates the hyperphagic and thermoregulatory responses to fasting and DIO in C57Bl/6J mice. Disruption of the fasting response in NPYko mice supports previous studies carried out in 129 and C57Bl/6J mice (3,8,9). We found that C57Bl/6J-NPYko showed greater weight loss during fasting and slower weight recovery during refeeding. Hyperphagia and reduction in energy expenditure typical of fasting were both attenuated by NPY deficiency. In contrast to wild-type C57Bl/6J mice, V_{O_2} level remained elevated in fasted NPYko mice and closely paralleled an increase in BAT UCP-1 expression. Interestingly, NPY deficiency did not affect body temperature during cold exposure, suggesting that NPY plays a critical role in the thermoregulatory response to fasting but not cold stress.

We examined hypothalamic neuropeptides that mediate energy balance to determine whether ablation of the *NPY* gene would alter their distribution and levels of expression (1). AGRP is normally coexpressed with NPY in the arcuate nucleus and acts as an endogenous antagonist of α-melanocyte stimulating hormone in the paraventricular nucleus (PVN), leading to stimulation of food intake, reduced thermogenesis, and weight increase (1). Arcuate NPY and AGRP neurons increase PVN expression of CRH and thyrotropin-releasing hormone, which are well known to inhibit appetite, stimulate thermogenesis, and decrease weight (1). An unsual characteristic of AGRP is that it has a prolonged stimulatory effect on food intake, possibly by interacting with syndecans (22,23). AGRP and NPY are both induced by fasting and mediate hyperphagia (1). POMC (a precursor of α-melanocyte–stimulating hormone) and CART are coexpressed in a separate population of neurons in the arcuate nucleus and act in the PVN and lateral hypothalamus to inhibit feeding, increase energy expenditure, and reduce weight (1). Leptin and insulin exert their anorexigenic actions partly by suppressing NPY/AGRP and increasing POMC/CART (1,24). Although POMC or CART ablation in mice produced the expected obese phenotype (19,25,26), earlier studies did not reveal significant effects of NPY and AGRP ablation on feeding or weight (2,27). However, recent studies have demonstrated crucial roles for NPY and AGRP (13,14). Ablation of NPY/AGRP had minimal effect on feeding in neonatal mice, whereas ablation of the same neurons caused severe starvation in adults (13,14). These results suggest that other neuronal networks may compensate for lack of NPY/AGRP in maintaining energy balance during early development but not in adults (13,14).

Ablation of a neuropeptide may alter the expression of other neuropeptides, as shown by an increase in NPY expression in the dorsomedial nucleus when the MC4 receptor is disrupted (28). We found that AGRP expression was restricted to the arcuate nucleus, but the levels were significantly increased in NPYko mice, suggesting a developmental compensation for NPY deficiency. On the other hand, the distributions and levels of POMC, CRH, and MCH were not altered in NPYko mice. Thus, we propose that the seemingly normal weight of NPYko mice under ad libitum–fed conditions is due at least in part to the orexigenic action of AGRP. However, AGRP failed to increase further during fasting in NPYko mice, which may explain why the postfast hyperphagia and weight recovery were attenuated in NPYko mice.

It was previously reported that female C57Bl/6J-NPYko mice developed a late-onset obesity on a normal chow diet (3). In contrast, we found no increase in weight in male or female NPYko mice. The reason for this discrepancy is unclear. Rather, C57Bl/6J-NPYko mice in the current study were resistant to DIO. NPYko mice consumed less high-fat diet and had higher energy expenditure than wild-type mice. Furthermore, nocturnal feeding was blunted in NPYko mice. The latter is consistent with another study where NPYko mice bred on the 129 strain had reduced nocturnal intake and consumed less amounts of a high-palatable diet (29). Thus, NPY appears to be essential for the circadian regulation of feeding and palatability cues (29). Resistance to DIO in C57Bl/6J-NPYko mice in the current study was associated with increased expression of POMC and CRH in hypothalamus, which are key neuropeptides implicated in appetite suppression and thermogenesis (1,30,31). Moreover, POMC and CRH increased in relation to adiposity in NPYko mice but fell in DIO wild-type mice. It has previously been reported that DIO in C57Bl/6J mice is associated with elevation of POMC levels in the hypothalamus (32). Our results suggest that the leaner phenotype of NPYko mice is partly mediated by the anorexigenic action of POMC and CRH.

This study highlights the importance of genetic background in obesity, diabetes, and lipid abnormalities (12,19,26,27). While the C57Bl/6J strain is highly susceptible to hyperphagia, obesity, insulin resistance, and diabetes, the 129 strain commonly used for gene knockout experiments is resistant to obesity (33–35). As we had predicted, the orexigenic action of NPY became apparent in the C57Bl/6J strain, which is susceptible to obesity. NPY deficiency blunted hyperphagia and weight gain in fasted and DIO C57Bl/6J mice. We propose that NPY is an important component of the "thrifty genotype" that optimizes energy storage. During fasting, NPY is likely to act in concert with other hypothalamic peptides to decrease energy expenditure, stimulate appetite, and replenish energy storage. This adaptive response signaled, at least in part, through falling levels of leptin and insulin and may have evolved as a protection against the threat of starvation (1,36). On the other hand, NPY could predispose to obesity in genetically susceptible individuals by stimulating food intake and reducing energy expenditure when food is abundant.

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REFERENCES

1. Flier JS: Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 116:337–350, 2004
2. Erickson JC, Clegg KE, Palmiter RD: Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381:415–421, 1996
3. Segal-Lieberman G, Trombly DJ, Juthani V, Wang X, Maratos-Flier E: NPY ablation in C57BL/6 mice leads to mild obesity and to an impaired

refeeding response to fasting. *Am J Physiol Endocrinol Metab* 284:E1131–E1139, 2003
4. Herzog H: Neuropeptide Y and energy homeostasis: insights from Y receptor knockout models. *Eur J Pharmacol* 480:21–29, 2003
5. Marsh DJ, Hollopeter G, Kafer KE, Palmiter RD: Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat Med* 4:718–721, 1998
6. Pedrazzini T, Seydoux J, Kunstner P, Aubert JF, Grouzmann E, Beermann F, Brunner HR: Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat Med* 4:722–726, 1998
7. Erickson JC, Hollopeter G, Palmiter RD: Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* 274:1704–1707, 1996
8. Bannon AW, Seda J, Carmouche M, Francis JM, Norman MH, Karbon B, McCaleb ML: Behavioral characterization of neuropeptide Y knockout mice. *Brain Res* 868:79–87, 2000
9. Sindelar DK, Mystkowski P, Marsh DJ, Palmiter RD, Schwartz MW: Attenuation of diabetic hyperphagia in neuropeptide Y–deficient mice. *Diabetes* 51:778–783, 2002
10. Sindelar DK, Ste Marie L, Miura GI, Palmiter RD, McMinn JE, Morton GJ, Schwartz MW: Neuropeptide Y is required for hyperphagic feeding in response to neuroglucopenia. *Endocrinology* 145:3363–3368, 2004
11. Sainsbury A, Bergen HT, Boey D, Bamming D, Cooney GJ, Lin S, Couzens M, Stroth N, Lee NJ, Lindner D, Singewald N, Karl T, Duffy L, Enriquez R, Slack K, Sperk G, Herzog H: Y2Y4 receptor double knockout protects against obesity due to a high-fat diet or Y1 receptor deficiency in mice. *Diabetes* 55:19–26, 2006
12. West DB, Boozer CN, Moody DL, Atkinson RL: Dietary obesity in nine inbred mouse strains. *Am J Physiol* 262:R1025–R1032, 1992
13. Luquet S, Perez FA, Hnasko TS, Palmiter RD: NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 310:683–685, 2005
14. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, Barsh GS, Horvath TL, Bruning JC: Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci* 8:1289–1291, 2005
15. Hahn TM, Breininger JF, Baskin DG, Schwartz MW: Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1:271–272, 1998
16. Takahashi N, Patel HR, Qi Y, Dushay J, Ahima RS: Divergent effects of leptin in mice susceptible or resistant to obesity. *Horm Metab Res* 34:691–697, 2002
17. Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS: Adiponectin acts in the brain to decrease body weight. *Nat Med* 10:524–529, 2004
18. Tritos NA, Elmquist JK, Mastaitis JW, Flier JS, Maratos-Flier E: Characterization of expression of hypothalamic appetite-regulating peptides in obese hyperleptinemic brown adipose tissue-deficient (uncoupling protein-promoter-driven diphtheria toxin A) mice. *Endocrinology* 139:4634–4641, 1998
19. Balthasar N, Coppari R, McMinn J, Liu SM, Lee CE, Tang V, Kenny CD, McGovern RA, Chua SC Jr, Elmquist JK, Lowell BB: Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* 42:983–991, 2004
20. Nagy TR, Clair AL: Precision and accuracy of dual-energy X-ray absorptiometry for determining in vivo body composition of mice *Obes Res* 8:392–398, 2000
21. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS: Role of leptin in the neuroendocrine response to fasting. *Nature* 382:250–252, 1996
22. Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, Van Der Ploeg LH, Woods SC, Seeley RJ: Long-term orexigenic effects of AgRP-(83–132) involve mechanisms other than melanocortin receptor blockade. *Am J Physiol Regul Integr Comp Physiol* 279:R47–R52, 2000
23. Reizes O, Lincecum J, Wang Z, Goldberger O, Huang L, Kaksonen M, Ahima R, Hinkes MT, Barsh GS, Rauvala H, Bernfield M: Transgenic expression of syndecan-1 uncovers a physiological control of feeding behavior by syndecan-3. *Cell* 106:105–116, 2001
24. Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL, Cone RD, Low MJ: Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 411:480–484, 2001
25. Challis BG, Coll AP, Yeo GS, Pinnock SB, Dickson SL, Thresher RR, Dixon J, Zahn D, Rochford JJ, White A, Oliver RL, Millington G, Aparicio SA, Colledge WH, Russ AP, Carlton MB, O'Rahilly S: Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3–36). *Proc Natl Acad Sci U S A* 101:4695–4700, 2004
26. Wierup N, Richards WG, Bannon AW, Kuhar MJ, Ahren B, Sundler F: CART knock out mice have impaired insulin secretion and glucose intolerance, altered beta cell morphology and increased body weight. *Regul Pept* 129:203–211, 2005
27. Qian S, Chen H, Weingarth D, Trumbauer ME, Novi DE, Guan X, Yu H, Shen Z, Feng Y, Frazier E, Chen A, Camacho RE, Shearman LP, Gopal-Truter S, MacNeil DJ, Van der Ploeg LH, Marsh DJ: Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of energy homeostasis in mice. *Mol Cell Biol* 22:5027–5035, 2002
28. Kesterson RA, Huszar D, Lynch CA, Simerly RB, Cone RD: Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of the agouti obesity syndrome. *Mol Endocrinol* 11:630–637, 1997
29. Sindelar DK, Palmiter RD, Woods SC, Schwartz MW: Attenuated feeding responses to circadian and palatability cues in mice lacking neuropeptide Y. *Peptides* 26:2597–2602, 2005
30. Yasuda T, Masaki T, Kakuma T, Yoshimatsu H: Hypothalamic melanocortin system regulates sympathetic nerve activity in brown adipose tissue. *Exp Biol Med (Maywood)* 229:235–239, 2004
31. Arase K, York DA, Shimizu H, Shargill N, Bray GA: Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats. *Am J Physiol* 255:E255–E259, 1988
32. Bergen HT, Mizuno T, Taylor J, Mobbs CV: Resistance to diet-induced obesity is associated with increased proopiomelanocortin mRNA and decreased neuropeptide Y mRNA in the hypothalamus. *Brain Res* 851:198–203, 1999
33. Coleman DL, Hummel KP: The influence of genetic background on the expression of the obese (Ob) gene in the mouse. *Diabetologia* 9:287–293, 1973
34. Colombo C, Haluzik M, Cutson JJ, Dietz KR, Marcus-Samuels B, Vinson C, Gavrilova O, Reitman ML: Opposite effects of background genotype on muscle and liver insulin sensitivity of lipoatrophic mice: role of triglyceride clearance. *J Biol Chem* 278:992–999, 2003
35. Goren HJ, Kulkarni RN, Kahn CR: Glucose homeostasis and tissue transcript content of insulin signaling intermediates in four inbred strains of mice: C57BL/6, C57BLKS/6, DBA/2, and 129X1. *Endocrinology* 14:3307–3323, 2004
36. Schwartz MW, Woods SC, Seeley RJ, Barsh GS, Baskin DG, Leibel RL: Is the energy homeostasis system inherently biased toward weight gain? *Diabetes* 52:232–238, 2003