Factors Contributing to Obesity in Bombesin Receptor Subtype-3-Deficient Mice


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Mice with a targeted disruption of bombesin receptor subtype-3 (BRS-3 KO) develop hyperphagia, obesity, hypertension, and impaired glucose metabolism. However, the factors contributing to their phenotype have not been clearly established. To determine whether their obesity is a result of increased food intake or a defect in energy regulation, we matched the caloric intake of BRS-3 KO mice to wild-type (WT) ad libitum (ad lib) fed controls over 21 wk. Although BRS-3 KO ad lib-fed mice were 29% heavier, the body weights of BRS-3 KO pair-fed mice did not differ from WT ad lib-fed mice. Pair-feeding BRS-3 KO mice normalized plasma insulin but failed to completely reverse increased adiposity and leptin levels. Hyperphagia in ad lib-fed KO mice was due to an increase in meal size without a compensatory decrease in meal frequency resulting in an increase in total daily food intake. An examination of neuropeptide Y, proopiomelanocortin, and agouti-related peptide gene expression in the arcuate nucleus revealed that BRS-3 KO mice have some deficits in their response to energy regulatory signals. An evaluation of the satiety effects of cholecystokinin, bombesin, and gastrin-releasing peptide found no differences in feeding suppression by these peptides. We conclude that hyperphagia is a major factor leading to increased body weight and hyperinsulinemia in BRS-3 KO mice. However, our finding that pair-feeding did not completely normalize fat distribution and plasma leptin levels suggests there is also a metabolic dysregulation that may contribute to, or sustain, their obese phenotype. (Endocrinology 149: 971–978, 2008)

Bombesin (BN)-like peptides represent a large family of amphibian and mammalian peptides that are biologically active in mammals (1–4). The mammalian members of this family, gastrin-releasing peptide (GRP) and neuropeptide B (NMB), have been found in visceral and central nervous system (CNS), and specific receptor subtypes corresponding to these peptides have been cloned and characterized (5, 6). More recently, a third mammalian BN receptor subtype, BN receptor subtype-3 (BRS-3), was identified using homology screening and found to share a high degree of structural similarity with GRP receptor (GRP-R) and NMB-R (51 and 47%, respectively) (4, 8). This receptor subtype was originally described in pregnant guinea pig uterus, human and guinea pig small-cell lung carcinomas, and secondary spermatocytes (9–11). Subsequently, BRS-3 mRNA expression was demonstrated in the mouse CNS primarily localized to the hypothalamus, most notably the paraventricular, dorsomedial, and arcuate nuclei (12, 13). Although the initial characterizations reported a limited distribution in the CNS, a subsequent immunohistochemical analysis using BRS-3 antisera demonstrated a much wider distribution, similar to that of GRP-R and NMB-R, with the highest densities of labeling in the cortex, hippocampus, hypothalamus, and thalamus (14).

Despite the close structural homology with other mammalian BN receptor subtypes, BRS-3 displays a pharmacological profile that is distinct from either GRP-R or NMB-R. Whereas BN binds with equal and high affinity to both GRP-R and NMB-R, it has a very low affinity for BRS-3 (15). There is currently no known naturally occurring high-affinity ligand for BRS-3, and thus it is classified as an orphan receptor.

Due to the lack of an identifiable ligand, information regarding a function for BRS-3 has been limited. In 1997, Ohki-Hamazaki and colleagues (13) developed a mouse lacking functional BRS-3 to determine a physiological and behavioral role for this BN receptor subtype. Mice deficient in BRS-3 developed obesity and hyperphagia beginning at approximately 16 wk of age that were accompanied by hypertension, disturbances in glucose metabolism, increased feeding efficiency, and lower fasting metabolic rate. BRS-3 knockout (KO) mice also exhibited an up-regulation of hypothalamic melanin-concentrating hormone receptor mRNA and an enhanced hyperphagic response to exogenous melanin-concentrating hormone administration (16). Together, these findings led to the conclusion that BRS-3 participates in food intake and energy homeostasis.

Although BRS-3 KO mice have significant abnormalities in food intake and body weight, the factors contributing to their phenotype have not been clearly identified. Previous studies have reported that pre-obese BRS-3 KO mice exhibit a modest

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Abbreviations: ad lib, ad libitum; AGRP, agouti-related peptide; ARC, arcuate nucleus; BN, bombesin; BRS-3, bombesin receptor subtype-3; CCK, cholecystokinin; CNS, central nervous system; CORT, corticosterone; GRP, gastrin-releasing peptide; GRP-R, GRP receptor; HPA, hypothalamic-pituitary-adrenal; KO, knockout; NMB, neuropeptide B; NPY, neuropeptide Y; POMC, proopiomelanocortin; WT, wild type. Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
decrease in oxygen consumption, suggesting that reduced metabolic rate may precede abnormalities in food intake and body weight. However, the extent to which metabolic alterations are secondary to increased food intake and body weight has not been established. Moreover, studies have not addressed whether the absence of BRS-3 leads to a disruption in other neuropeptide systems that regulate energy balance or whether BRS-3 deficiency affects the response to peptides that participate in short-term satiety signaling. In the present study, we have addressed these questions by 1) determining the effects on body weight gain and hypothalamic gene expression of energy regulatory peptides in BRS-3 KO mice that were pair-fed the amount of food consumed by ad lib-fed wild-type (WT) mice, 2) characterizing patterns of food intake in KO and WT mice to determine which meal parameters are altered in KO mice that contribute to their hyperphagia, and 3) evaluating the effects of the meal-related satiety peptides cholecystokinin (CCK), GRP, and BN on food intake in KO and WT mice.

Materials and Methods

Generation of BRS-3-deficient mice

Male BRS-3-deficient mice and WT littermates were generated at The National Institutes of Health, Bethesda, MD, by targeted mutagenesis in embryonic stem cells. Homologous recombination resulted in disruption of the BRS-3 coding region, residing on the X chromosome, by replacement of exon 2 with *Neo* sequence (Fig. 1A). Correctly targeted embryonic stem cell clones were identified by Southern analysis with a 5′-flanking probe and by long-range PCR at the 3′ junction. Germline transmission was confirmed by Southern analysis (Fig. 1B) where a 7.5-kb *PstI* fragment was detected from the mutant allele in contrast to the 16-kb fragment detected from the WT allele. Offspring were subsequently genotyped by PCR (Fig. 1C) using oligo pairs that distinguished the WT from the mutant allele. Loss of BRS-3 expression was confirmed by RT-PCR analysis of brain mRNA (Fig. 1D). A 5′-flanking probe (black box in A) identifies the 16-kb WT and 7.5-kb recombinant *PstI* fragments. C, Genotyping of offspring by PCR. The WT oligo pair amplifies a 390-bp fragment, and the recombinant oligo pair amplifies a 165-bp fragment. Shown are representative offspring: +/+BRS-3 female (lane 1), +/+ female (lane 2), +/+ male (lane 3), and BRS-3/Y male (lane 4). A 450-bp fragment is amplified from +/+ female (lane 1), +/+ female (lane 2), +/+ male (lane 3), and BRS-3/Y male (lane 4).

Food intake

Pair-feeding. Eleven male BRS-3 KO mice and 10 WT littermates were the subjects of this experiment. Males were used for this study because they exhibit a more pronounced phenotype than females and are less likely to exhibit fluctuations in food intake due to hormonal influences. Mice were housed individually in Plexiglas cages (8 × 8 in.) with a wire-mesh bottom equipped with a nesting box. The cages were housed in a temperature-controlled room on a 12-h light, 12-h dark cycle. Tap water was available ad lib (ad lib, diet; Bio-serv, Frenchtown, NJ) was provided in the manner indicated below.

Food intake and body weights were measured once a week beginning at 9 wk of age and compared between groups. When food consumption by the KO mice was significantly greater (P < 0.05) than that of the WT mice, the mice were divided into four experimental groups as follows: 1) WT mice given *ad lib* food access (*n* = 6, *WT ad lib*), 2) KO mice given *ad lib* food access (*n* = 6, KO *ad lib*), 3) KO mice calorically matched to the amount of food consumed by the WT *ad lib* group (*n* = 5, KO pair-fed), and 4) WT mice given restricted food access (*n* = 4, WT restricted). The amount of restriction was based on the amount consumed by the WT *ad lib* group reduced by the same percent decrease that was imposed on the KO pair-fed group compared with the KO *ad lib* group.

Food intake was monitored daily for the duration of the experiment. The average daily food intake for the WT *ad lib* and KO *ad lib* groups was determined on a weekly basis, and adjustments to the amount of food given to either the KO pair-fed or WT restricted groups were made as necessary. Body weights were recorded once a week throughout the experiment (32 wk of age).

Meal pattern analysis. At the conclusion of the pair-feeding experiment, an analysis of meal patterns was conducted in the WT *ad lib* and KO *ad lib* groups. Mice were moved from their home cages and maintained in 8 × 8 in. Plexiglas cages containing a nest box and an opening to a food bin attached to a pellet dispenser (Coulbourn Instruments, Allentown, PA). The food bin was equipped with an infrared device that detected when a 20-mg pellet (Bio-serv rodent grain-based diet) was present in the feeder. Each feeder was connected to a computer interface that recorded the number of pellets that were removed from the bin at 5-min intervals (Med Associates, Inc. Software, St. Albans, VT). Meal criteria were set for at least five pellets followed by 10 min with no feeding. Mice were acclimated to the feeding apparatus for at least 7 d before data collection was initiated to assure that food intake was consistent and spillover was minimal. Data for total food intake, meal size, and meal frequency (number of meals) were averaged for 3 d, and each parameter was compared between WT and KO mice.

Blood and fat analysis

At the conclusion of the meal pattern analysis, mice were returned to their home cages and maintained for 2 wk as described above before being killed. They were then food deprived for 17 h and anesthetized with ether, and blood was collected from the retroorbital sinus for determination of plasma insulin, leptin, and corticosterone (CORT). To evaluate whether changes in these measurements occur early in life,
additional groups of seven WT and seven KO male mice that were 10 wk of age were also included. All blood analyses were performed by the Diabetes Research Center at The University of Pennsylvania Medical Center.

To determine fat distribution, white adipose tissue from the sc (left side only) and epididymal fat pads and brown adipose tissue from the intrascapular fat depot were dissected, and wet weight was assessed. Tissue weights were compared between groups at each site.

**In situ hybridization**

Immediately after blood was collected for hormonal measurements, mice were rapidly decapitated and the brains removed and flash frozen in isopentane chilled on dry ice. To evaluate early-onset changes in hypothalamic gene expression, another set of 9-wk-old WT (n = 7) and KO (n = 7) mice that did not differ in body weight were processed in the same manner. Brains were sectioned on a cryostat in the coronal plane at 14 μm, mounted onto SuperFrost microscope slides (Fisher Scientific, Pittsburgh, PA), fixed with 4% paraformaldehyde, and stored at −80 °C until ready for use.

For in situ hybridization, tissue sections were brought to room temperature and treated with acetic anhydride and ethanol. Riboprobes from cDNA templates for neuropeptide Y (NPY), proopiomelanocortin (POMC), and agouti-related peptide (AGRP) were transcribed with the appropriate polymerases in the presence of [35S]UTP and purified using STE select-D columns (Eppendorf-5 Prime, Gaithersburg, MD) to yield a specific activity of 5 × 107 cpm/μg. Tissue sections from the arcuate nuclei (ARC) were hybridized for NPY, POMC, and AGRP mRNAs using procedures previously described (17). The hybridization signal in the ARC of each brain section was quantified from digitized autoradiograms using National Institutes of Health Scion Image software. Values for each animal were determined from three sections and expressed as the percentage of the mean value of the WT control mice.

**Response to peripheral peptide administration**

An additional cohort of six male KO (starting weight = 24.3 ± 0.4 g) and six male WT (starting weight = 27.6 ± 0.6 g) mice were used for this experiment over the course of 10 wk. The mice were maintained in the same manner as described previously. To test the effects of several orexigenic peptides that have been shown to participate in meal-related food intake, mice were given an ip injection (1 ml/100 g body weight) of either 0.9% saline or a range of doses of CCK, BN, and GRP18–27, the biologically active portion of GRP (Bachem, Torrance, CA). There was at least 48 h between peptide injections, and every mouse received the full dose range for each peptide before beginning the next series. The order of testing was CCK, BN, and GRP18–27. Immediately after the injection, the mice were presented with a 0.5 kcal/ml glucose solution in calibrated tubes, and intake was recorded 30 min later to the nearest 0.1 ml. Testing was conducted during the light portion of the light-dark cycle 1 h before lights out.

**Data analysis**

Statistical analyses were conducted with SigmaStat software using one-way ANOVA, two-way repeated-measures ANOVA, or Student's t test where appropriate. Subsequent comparisons between groups were carried out using Newman-Keuls test for pairwise multiple comparisons. Differences were considered statistically significant if P < 0.05.

**Results**

**Effects of pair-feeding on body weight**

The body weight data for the four experimental groups from age 9–32 wk is shown in Fig. 2. The overall ANOVA indicated a significant group effect on body weight (F3,37 = 36.6; P < 0.001). Post hoc analyses revealed that body weights for the KO ad lib group (30.4 ± 0.7 g) were significantly greater than the WT ad lib group (25.7 ± 0.5 g) beginning at 13 wk of age and continued for the duration of the experiment. By 32 wk of age, KO ad lib mice had a mean body weight that was 28.6% above that of the WT ad lib group (45.4 ± 1.1 g for the KO ad lib vs. 32.4 ± 1.1 g for the WT ad lib).

With the exception of wk 25, pair-feeding the KO group to the caloric intake of the WT ad lib group normalized body weight such that the KO pair-fed group did not differ significantly from the WT ad lib group (P > 0.05). The WT restricted group weighed significantly less (P < 0.001) than the WT ad lib group beginning at 16 wk of age with an ending body weight that was 19.7% lower than WT ad lib mice.

A comparison of the amount of food consumed per gram of body weight between BRS-3 KO ad lib and WT ad lib mice at 12, 20, and 28 wk is shown in Fig. 3. The early time point (12 wk) showed no significant difference in this ratio between the two groups (P > 0.05). However, as body weight differences between ad lib-fed KO and WT mice continued to increase, less food was consumed relative to body weight in the BRS-3 KO ad lib group at both 20 and 28 wk (P < 0.05).
Food intake and meal pattern analysis

Average daily food intake for the experimental groups is shown in Fig. 4. In agreement with previous reports, KO ad lib mice exhibited higher food intake than WT ad lib mice over the course of the experiment (P < 0.001). In accordance with our experimental design, food consumption between KO pair-fed and WT ad lib mice was not significantly different (P > 0.05), and intake in WT restricted mice was significantly lower than WT ad lib mice (P < 0.001).

Meal pattern results revealed that the total number of pellets consumed in 24 h was greater in KO ad lib mice (219.9 ± 12.7) compared with WT ad lib mice (176.7 ± 5.8) (P = 0.01). Although the number of meals taken in 24 h was not different between KO and WT mice (14.8 ± 1.3 vs. 15.9 ± 1.1, respectively, P = 0.54), there was a significant increase in meal size (number of pellets) in KO ad lib mice (14.8 ± 1.4) compared with WT ad lib mice (9.8 ± 0.5) (P = 0.007). The increased meal size, without a change in meal frequency, accounted for the increase in total pellet intake over 24 h.

Blood and fat analysis

Epididymal, sc, and brown fat depots (Fig. 5) and measurements of insulin, leptin, and CORT (Table 1) were evaluated in mice that were 10 wk of age and in the animals from the pair-feeding experiment at 36 wk of age. At 10 wk of age, KO mice in this group were significantly heavier compared with WT mice (26.1 ± 0.5 g vs. 23.8 ± 0.6 g, P = 0.01). The increase in body weight in KO mice was accompanied by a significant increase in both sc (KO = 151 ± 17.2 mg; WT = 95 ± 10.3 mg; P = 0.02) and epididymal fat (KO = 247.4 ± 29.1 mg; WT = 141.3 ± 18.4 mg) but not in brown fat (KO = 78.6 ± 7.6 mg; WT = 72.7 ± 13.3 mg; P = 0.5). The increase in sc and epididymal fat depots corresponded with elevated plasma leptin in the KO mice (P < 0.001).

At the conclusion of the experiment (36 wk of age), there was a significant effect on the amount of epididymal fat (F3,17 = 23.85; P < 0.001) between groups. As mentioned above, KO pair-fed mice exhibited increased levels of epididymal fat that were reflected in an increase in leptin levels compared with the WT ad lib group (P < 0.001).

KO ad lib mice also had significantly elevated levels of insulin compared with WT ad lib mice (P < 0.05). Although KO pair-fed mice had increased adiposity and plasma leptin levels, pair-feeding normalized insulin levels to that of WT ad lib mice (P < 0.05).

There were no differences in CORT levels between WT and KO mice at 10 wk of age (P > 0.05). By 36 wk of age, WT restricted mice exhibited increased CORT levels compared with all other groups (P < 0.05). There was no difference in plasma CORT among WT ad lib, KO ad lib, and KO pair-fed mice (P > 0.05).

Patterns of hypothalamic gene expression in BRS-3 WT and KO mice

To determine whether early differences in ARC POMC, NPY, and AGRP gene expression may contribute to the development of the obese phenotype in KO mice, we compared mRNA levels of these peptides in WT and KO mice at 9 wk of age before weight differences between the two groups (WT = 19.5 ± 0.7 g and KO = 20.4 ± 0.4 g). There were no significant differences in ARC POMC, NPY, or AGRP mRNA levels between WT and KO mice at this age (P > 0.05).

Expression of POMC, NPY, and AGRP mRNA in 36-wk-old WT ad lib, WT restricted, KO ad lib, and KO pair-fed mice is shown in Fig. 6. Levels of POMC mRNA in the ARC nucleus did not differ across treatment groups (F3,17 = 0.48; P = 0.69). However, a significant group effect was observed for ARC NPY (F3,17 = 6.84; P = 0.003) and AGRP (F3,17 = 10.52; P < 0.001). Post hoc analyses revealed that NPY gene
expression was significantly higher in the WT restricted group compared with all other groups (P < 0.02) with an increase of 45% above that of WT ad lib mice. KO mice that were pair-fed to the amount of food consumed by WT ad lib mice showed no differences in ARC NPY compared with WT ad lib and the KO ad lib (P > 0.05). AGRP mRNA levels were significantly decreased in KO ad lib mice compared with WT ad lib mice (P < 0.05). Pair-feeding normalized levels of AGRP gene expression such that no differences were observed between KO pair-fed and WT ad lib mice (P > 0.05).

**Feeding response to exogenous peptide administration**

As shown in Fig. 7, peripheral administration of CCK resulted in significant decreases in 30-min glucose intake in both BRS-3 KO and WT mice. The overall ANOVA revealed a significant main effect of dose (P < 0.001) but no difference between groups or interaction (P > 0.05).

Like CCK, BN and GRP also inhibited glucose intake in both BRS-3 KO and WT mice. Overall ANOVA after BN administration indicated there was no significant difference in intake between groups (F1,10 = 0.62; P = 0.45), but there was a significant dose effect (F10,4 = 26.119; P < 0.001). Similar to BN, glucose intake after GRP did not differ between groups but produced a significant dose effect (F10,4 = 9.113; P < 0.001).

**Discussion**

The orphan mammalian BN receptor subtype, BRS-3, has been implicated in the control of food intake and energy balance. Studies have demonstrated that mice with a targeted disruption of BRS-3 develop hyperphagia, obesity, and impaired glucose metabolism. Moreover, BRS-3 mRNA is expressed...
pressed in several hypothalamic nuclei that are important in energy homeostasis.

In the present study, we sought to identify the factors that contribute to the phenotypic characteristics of BRS-3 KO mice. In confirmation of previous reports, ad lib-fed BRS-3 KO mice exhibited hyperphagia and increased body weight compared with ad lib-fed WT mice. However, our data clearly demonstrate that the increase in body weight in KO mice can be overcome by matching their caloric intake to that of wild-type controls. Although body weight and insulin levels were normalized by pair-feeding, there remained a significant increase in both leptin levels and body fat distribution that confirms a metabolic dysregulation in BRS-3 KO mice. These data extend previous results demonstrating a small but significant decrease in oxygen consumption in BRS-3 KO mice before obesity onset (13).

The analysis of body fat indicated that ad lib-fed BRS-3 KO mice exhibited elevations in epididymal, sc, and brown fat depots. Whereas pair-feeding normalized levels of sc and brown fat, it failed to normalize levels of epididymal fat. Numerous studies have shown that adipose tissue displays different characteristics based on its anatomic location (18, 19). For example, early work had demonstrated that sc fat had lower levels of basal and insulin-stimulated glucose uptake than epididymal fat (20). Regional differences in lipolytic activity of isolated fat cells have also been reported, with epididymal fat exhibiting increased rates of lipolysis and a greater lipolytic response to catecholamines and \( \beta \)-adrenergic receptor agonists than sc fat. These differences are thought to result from greater numbers of \( \beta \)-adrenergic receptors and a lower \( \alpha_2 \)/\( \beta_3 \)-adrenergic receptor ratio in this tissue (21). Thus, our finding that pair-feeding did not completely reverse the increased levels of epididymal fat in BRS-3 KO mice may reflect an abnormality in regional fat cell metabolism in these animals. Although elevated leptin levels in pair-fed BRS-3 KO mice may be directly related to the increased amount of epididymal fat, we also cannot rule out the possibility that these animals have impaired leptin secretion or production.

Studies to elucidate potential mechanisms for obesity in BRS-3 KO mice have been conducted by crossing BRS-3 KO mice with KK-Ay mice, an obese mouse model that exhibits hyperphagia and hyperinsulinemia due to ectopic expression of agouti protein, an antagonist at the melanocortin 4 receptor (22, 23). Hybrid mice (i.e. Ay mice without BRS-3) were significantly heavier than either genotype alone, displayed insulin levels that were 2.3-fold higher than those with just the Ay gene, and showed impairments in glucose-induced insulin secretion implicating BRS-3 in the regulation of plasma insulin concentration and sensitivity. In the present study, we found that pair-fed KO mice had insulin levels that were comparable to ad lib-fed WT mice, suggesting that hyperinsulinemia is not a primary deficit in ad lib-fed KO mice but is secondary to their increased body weight. This plasma profile is in contrast to pair-fed ob/ob mice that have been shown to have equivalent weight gain to lean mice but continue to exhibit elevated insulin and CORT levels (24).

Our data also demonstrate that differences in plasma CORT levels were apparent only in the WT restricted group. This finding is in agreement with numerous studies in rodents showing that food restriction activates the hypothalamic-pituitary-adrenal (HPA) axis resulting in an elevation in plasma CORT (25, 26). Although the KO pair-fed group was equally food restricted compared with KO ad lib mice, they failed to exhibit elevated plasma CORT levels. This result suggests that either BRS-3 KO mice have deficits in HPA activation or that food restriction is not perceived as a stressor in these animals. The possibility of HPA axis dysregulation has been suggested by previous studies demonstrating a decrease in anxiety-related behaviors in BRS-3 KO mice. When anxiety levels were assessed in an elevated plus maze, KO mice showed less anxiety than WT controls as well as a decrease in the behavioral response to social isolation and a novel environment (27–29).

Results from the meal pattern analysis revealed that a primary deficit contributing to the obese phenotype of BRS-3 KO mice is an uncompensated increase in meal size that resulted in an overall increase in total food intake. This pattern of food intake has previously been described in other animal models of obesity. For example, the ob/ob mouse and Zucker (fa/fa) rat, both of which have deficits in leptin signaling, exhibit hyperphagia that is attributed to increased meal size primarily in the dark phase of the light-dark cycle (30–32). Increased meal size has also been reported in the Otsuka Long-Evans Tokushima Fatty rat, an obese animal model that lacks the receptor for CCK, a meal-related satiety peptide (33).

The increased meal size suggested the possibility that deficits in meal-related satiety signaling may contribute to hyperphagia in BRS-3 KO mice. Thus, we tested the ability of the peptides CCK, BN, and GRP to reduce food intake in a short-term feeding test using a palatable test meal. These peptides are released in response to a meal and have been shown to suppress short-term food intake when given exogenously in a manner consistent with naturally occurring satiety (34). Although previous studies have reported an increased responsiveness to sweet taste in BRS-3 KO mice (35), we did not observe any differences in baseline glucose intake between KO and WT mice during our short-term feeding test. Our finding that there was no difference between BRS-3 KO and WT mice in their response to exogenously administered CCK, BN, and GRP suggests that intact BRS-3 is not required for feeding inhibition by these peptides. Although BRS-3 is structurally homologous to GRP-R and NMB-R, the result that BN and GRP were equally effective in suppressing food intake in KO and WT mice suggests that exogenously administered BN and GRP are not acting as ligands at this receptor to produce satiety.

To determine whether lack of functional BRS-3 could lead to developmental changes in critical energy regulatory circuits that would predispose animals to hyperphagia and obesity, analysis of gene expression of NPY, POMC, and AGRP in BRS-3 KO and WT mice was conducted at 9 wk, before body weight differences. Early-onset alterations in hypothalamic NPY gene expression have been proposed as a potential mechanism for the etiology of hyperphagia and obesity in the Otsuka Long-Evans Tokushima Fatty and Zucker (fa/fa) rat (17, 36). Our finding that POMC, NPY, and AGRP mRNA expression levels were not different between KO and WT mice at 9 wk suggests that innate deficits

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in these signaling pathways do not contribute to driving hyperphagia in BRS-3 KO mice.

Another mechanism that could lead to dysregulated food intake or energy balance is the failure of critical hypothalamic signaling pathways to respond appropriately to changes in energy stores. This situation has been demonstrated in the obese Zucker rat that exhibits chronically elevated levels of hypothalamic NPY in the face of increased food intake and body weight but shows no significant change in NPY after food deprivation (36).

Numerous studies have identified the response of hypothalamic neuropeptide pathways to changes in energy status (7). An important site of action for the circulating adiposity thalamic neuropeptide pathways to changes in energy status (36).

In the present study, we found that WT restricted mice initiate a normal counterregulatory response to chronic food restriction by significantly elevating NPY gene expression. In contrast, BRS-3 KO mice failed to increase NPY mRNA levels in response to fasting and increased by leptin replacement. The net effect is that NPY promotes food intake and decreases energy expenditure, and POMC reduces food placement. The net effect is that NPY promotes food intake and increases energy expenditure.

Although activity levels and thermogenesis do not differ between BRS-3 KO and WT mice, previous data have shown that food restriction resulted in a slower rate of weight loss in BRS-3 KO mice than in WT mice, suggesting that BRS-3 KO mice have increased feed efficiency (13). In agreement with this result, our data demonstrated that as obesity progressed in BRS-3 KO mice by pair-feeding did not affect NPY gene expression. These findings suggest that BRS-3 KO mice may have deficits in NPY signaling that contribute to, or sustain, hyperphagia and obesity. Because both ad lib and pair-fed BRS-3 KO mice showed elevated plasma leptin levels, it is possible they develop leptin insensitivity that results in a failure to respond appropriately to metabolic signals. Consistent with this possibility, obese BRS-3 KO mice exhibit an attenuated response to the anorexigenic effects of centrally administered leptin (16).

In summary, we have demonstrated that pair-feeding BRS-KO mice to the caloric intake of ad lib WT mice over an extended period of time prevents body weight gain and hyperinsulinemia but fails to completely reverse increased levels of adiposity and plasma leptin. The obesity in ad lib-fed BRS-3 KO mice is, in part, attributed to a failure to compensate for increased meal size and to initiate normal responses in hypothalamic signaling pathways related to energy homeostasis. The behavioral and metabolic phenotype of BRS-3 KO mice differs from that of several well-established mouse models of obesity. As such, they provide a unique model to understand the role of this receptor in the complex interactions between food intake and energy balance.

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