Ghrelin and PYY in the regulation of energy balance and metabolism: lessons from mouse mutants

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Abstract

Ghrelin and peptide tyrosine-tyrosine (PYY) are gut hormones involved in the regulation of energy homeostasis but have been reported to exert opposing actions. Since their discovery, numerous functions have been assigned to ghrelin and PYY on the basis of in vitro experiments, in vivo pharmacological studies, and clinical studies. More recently, targeted mouse mutagenesis has been successfully applied to define the physiological role of these gut hormones. Transgenic overexpressors with an exogenous allele of the respective gene, and knockout mice (for ghrelin and GHSR), and GOAT-deficient or -overexpressor [growth hormone secretagogue receptor-1a (GHSR1a)]-deficient mice, double-knockout mice (for ghrelin and GHSR), and GOAT-deficient or -overexpressor mice, as well as mice deficient for PYY or neuropeptide Y receptors have allowed better definition of the actual physiological functions of ghrelin and PYY. This review summarizes findings from mutant mouse studies with emphasis on respective gene knockout and transgenic animals and describes how these studies contribute to the current understanding of how endogenous ghrelin and PYY as two major representatives of endocrine gut-brain communications may regulate energy and glucose homeostasis.

Ghrelin and PYY, Two Gut Peptides with Opposing Actions

Ghrelin, the only known circulating orexigenic factor, is a 28-amino acid peptide produced primarily in the stomach (59); however, small concentrations are synthesized in the small intestine, liver, pancreas, kidney, lung, pituitary, hypothalamus, placenta, and testes (28, 46, 60, 75, 123–125). At the time of its discovery in 1999, ghrelin was known to stimulate the release of growth hormone (GH) from the pituitary following release of growth hormone (GH) from the pituitary following a meal. It was therefore proposed that ghrelin triggered especially from experiments using genetically engineered mice.

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Ghrelin and PYY: Current Evidence from Mouse Mutagenesis

The development of mutant mouse models with gain or loss of function provides a key tool to study the physiological roles of hormones such as ghrelin and PYY. The following section will summarize recent data generated from genetically mutated mice with modifications in the expression of both ghrelin and PYY or alteration of their respective receptor activation.

Ghrelin deficiency results in a moderate metabolic phenotype. Single gene deletions of mouse ghrelin (30, 113, 127, 128) or the ghrelin receptor GHSR were generated by a number of different laboratories (2, 115, 135). In addition, our laboratory recently generated a mouse line deficient in both ghrelin and its receptor, resulting in a phenotype that was more profound than either mutant separately (92). Besides, Sun et al. (114) crossed leptin deficient (ob/ob) mice with mice deficient in ghrelin to study the interaction of ghrelin and leptin signaling. Moreover, two recent studies described a specific impairment of GHSR activation in the hypothalamus of rats (109) and 2) transgenic overexpression of ghrelin driven by the neuron-specific enolase promotor in mice (96). Such modulation of ghrelin function in the hypothalamus, a well-known central regulator of energy homeostasis, provides useful knowledge to dissect central versus peripheral actions of ghrelin.

Total plasma ghrelin levels are elevated during chronic states of energy deficiency, including cachexia and anorexia nervosa, and are depressed during conditions of energy excess such as obesity, although mass spectrometry-based confirmation of such immunoreactivity-based data is still missing. On the basis of pharmacological evidence, it was hypothesized that ghrelin-deficient mice would be hypophagic and leaner than their wild-type (WT) littermates. However, the first ghrelin-deficient mice (ghrelin−/−) generated by Sun et al. (113) and Wortley et al. (127) failed to demonstrate significant differences in body weight, body fat, or food intake compared with WT littermates when fed a regular chow diet. It was therefore speculated that, within a large redundant orexigenic signaling system, additional food intake-promoting system(s) were present that could compensate for the lack of ghrelin. In fact, such compensation may have occurred during early development, possibly via enhanced activity of several hypothalamic orexigenic neurons (127). However, changes in basal gene expression of orexigenic neuropeptides, agouti-related protein, melanin-concentrating hormone, proopiomelanocortin and NPY were not evident (127). Sun et al. (113) therefore deduced that ghrelin was not necessarily associated with any of the tested orexigenic neuropeptides. However, when Wortley et al. fed ghrelin−/− mice high-fat diet (HFD) immediately after weaning, a clear metabolic phenotype emerged (127). Despite having the same body weight and food intake, ghrelin−/− mice had a significantly lower respiratory quotient (RQ), reflecting marked differences in metabolic fuel preference. In a separate experiment, Wortley et al. (128) found that when young ghrelin−/− mice were exposed to an HFD they gained less weight and had reduced body fat content despite consuming similar amounts of food compared with WT mice. In addition, ghrelin−/− mice on HFD showed increased energy expenditure and locomotor activity, providing a potential mechanism for a difference in fat mass gain on HFD (128). These results clearly demonstrated that ghrelin does play an important role in regulating food intake that cannot be fully compensated for by other factors, at least when metabolically challenged with an HFD.

Since ghrelin−/− mice gained less weight during HFD feeding, Sun et al. (114) sought to determine whether the hyperphagia-induced obese phenotype of leptin deficient ob/ob mice could be ameliorated in the absence of ghrelin. Due to the effects of ghrelin on the induction of food intake and promo-
of fat storage, which suggested that ghrelin and leptin might be direct endogenous opponents in the control of energy balance, it was hypothesized that the absence of ghrelin might balance the fat deposition that occurs during leptin deficiency. However, ghrelin \(^{-/-}\)/lept \(^{ob/ob}\) double mutant mice had the same food intake, body weight, and body fat content as ob/ob mice, indicating that the absence of ghrelin does not affect the obese phenotype associated with leptin deficiency.

In summary, deleting the ghrelin gene does not seem to affect food intake, indicating that endogenous ghrelin is likely not an essential meal initiator or the key hormone to cause increased caloric ingestion in obesity. Also, deletion of the ghrelin gene in mice does not induce a lean or small phenotype, at least not on a regular chow diet. Moreover, the absence of orexigenic ghrelin is not sufficient to counteract the development of severe obesity observed in leptin-deficient mice. Nevertheless, feeding mice an HFD creates a condition in which ghrelin alters fat deposition and metabolism by decreasing body weight gain and fat mass, possibly by decreasing fuel efficiency and increasing fat oxidation. It therefore seems likely that the main role of endogenous ghrelin in the control of energy metabolism is that of a specific link between (certain components of) HFD and predominantly metabolic (rather than feeding-related) changes that overall result in altered lipid storage efficiency.

**Ghrelin receptor deficiency provides some protection against diet-induced obesity.** The physiological function of ghrelin requires the activation of its only known receptor, GHSR1a. Ablation of GHSR completely silences both ligand-induced and constitutive activity.

GHSR-deficient mice were again first generated by Sun et al. (115) and were found to have a normal growth rate and no differences in food intake compared with controls. Zigman et al. (135) exposed GHSR-deficient mice chronically to HFD and reported that mice deficient for the ghrelin receptor were protected from diet-induced obesity, similar to ghrelin-deficient mice and reported that mice deficient for the ghrelin receptor were protected from diet-induced obesity, similar to ghrelin-deficient mice and GHSR-deficient mice. After 19 weeks on HFD, female GHSR-deficient mice weighed 10.9% less and stored 16.5% less fat (128). A recent study by Longo et al. (70) confirms these findings, showing that GHSR-deficient mice have lower body weight and fat mass than WT littermates.

GHSR is expressed in a variety of peripheral tissues as well as various brain areas, most importantly in the arcuate nucleus, localized within the hypothalamus (47, 134). This region is central to energy homeostasis regulation, receiving, integrating, and processing neural, metabolic, and hormonal signals from the periphery and the CNS. Interestingly, recent evidence also suggests that ghrelin is synthesized by neurons within the arcuate nucleus (74); the physiological importance of such central ghrelin expression, however, remains unclear. To evaluate the specific function of GHSR in the hypothalamus, Shuto et al. (109) expressed GHSR-specific antisense RNA under the control of tyrosine hydroxylase, presumably, in the arcuate nucleus of rats. These transgenic rats showed a decreased number of GHSR-positive neurons in the arcuate nucleus and were hypophagic, smaller, and leaner than control rats. Decreased c-fos staining in hypothalami of GHSR KO mice that were fed a scheduled meal paradigm further suggested that ghrelin action in the hypothalamus augments arousal and locomotor activity in anticipation of a meal (14). Such food-anticipatory behavior might be controlled by the circadian clock proteins PER1 and PER2, which are coexpressed with ghrelin in stomach oxyntic glands. PER proteins might thereby serve as food-entrainable oscillators that control ghrelin expression and the systemic ghrelin output prior to feeding (67).

In summary, the deletion of the ghrelin receptor (GHSR) in female and male rodents partially protects from diet-induced obesity by decreasing fat deposition during chronic exposure to HFD. Different from ghrelin ligand-deficient mice, this appears primarily due to a reduction in food intake and food-anticipatory behavior, strongly suggesting that GHSR signaling contributes to physiological regulation of energy homeostasis. Similar to ablation of ghrelin, GHSR deficiency results in lower body weight and body fat. However, different from ghrelin KO mice, GHSR KO mice retain similar fuel efficiency despite decreased food consumption. The endogenous ghrelin-GHSR signaling pathway, therefore, interacts not only with hunger- and satiety-influencing neurons in the brain but also with systems that determine energy expenditure, deposition, and metabolic fuel preference, possibly via the central melanocortin system (84). It is still unclear where the switch from impacting either the feeding or the energy metabolism/nutrient partitioning pathway is, how it works, and if it involves a second ligand and/or a second receptor.

Interestingly, rare mutations in the human GHSR gene (88, 126) result in a loss of its constitutive activity. Investigations of two Moroccan families carrying a GHSR mutation reported impaired growth and, surprisingly, an obese rather than a lean phenotype (88). This may be explained by the fact that this mutation exclusively impairs the receptor’s constitutive activity but does not affect its acute response to ghrelin (54, 88). A recent case report on a patient with recessive partial isolated GH deficiency due to GHSR mutations corroborated the growth delay but also revealed a reduced body mass index. In vitro studies suggested that the mutations resulted in a partial loss of constitutive GHSR activity, but cell surface expression and the physiological response to ghrelin were unaffected. Thus, unlike in GHSR-deficient mice, the ghrelin-mediated receptor signaling remains functional in these characterized human mutants. On the basis of the human data, the impact of GHSR signaling on body growth may be highly influenced by the receptor’s constitutive activity.

Recent results obtained in our laboratory from the study of mice deficient for both ghrelin and the ghrelin receptor (GHSR) indicate that a complete functional absence of ghrelin signaling is sufficient to decrease body weight and fat mass and increase energy expenditure even when the mice are fed a chow diet (92). The metabolic phenotypes of mice with single deficiencies for either ghrelin or GHSR compared with double-KO (dKO) mice revealed a phenotype more severe than would be expected from the single-KO mice alone. This exacerbated phenotype of dKO mice suggests that additional ligands for the GHSR and additional receptors for ghrelin might exist. Such additional ligands and receptors could be deprived of their essential signaling counterpart when ghrelin and GHSR are both removed. That might be one explanation why a dKO aggravates the metabolic consequences of ghrelin.
or GHSR deficiency, respectively. Further research will be needed to confirm the existence and identify the additional components of such convergent signaling pathways. Such dissection will get us closer to clarifying the role of ghrelin signaling in the regulation of body weight and energy homeostasis.

**Novel models to study ghrelin physiology: GOAT overexpressor and KO mice.** One step toward better understanding of ghrelin-GHSR signaling is the very recent discovery of the ghrelin octanoyl acyltransferase GOAT (50, 132). Gutierrez et al. (50) thereby generated the first GOAT KO mouse model and convincingly demonstrated that GOAT is the only enzyme that can acylate serine-3 with an octanoyl side chain. GOAT expression in mice is highest in stomach and intestine but can also be found in pituitary and hypothalamus (42) and several peripheral tissues such as adrenals, white adipose tissue, or muscle (44). A recent report even suggests the possibility that GOAT can be found in circulation (112). Little information is yet available on GOAT regulation; after prolonged fasting, stomach GOAT mRNA levels were reported to be downregulated (58), or increased (42). Chronic food restriction may increase GOAT expression (44). Protein levels in stomach were recently reported to increase with fasting (112). Stomach GOAT expression did not differ between diet-induced obese (DIO) and lean chow-fed mice or between WT and leptin-deficient ob/ob mice (42, 58) but increased after administration of leptin (44).

When fed a diet rich in medium-chain fatty acids, GOAT-deficient mice had lower body weights and fat mass and higher energy expenditure while food intake was unchanged (58). In contrast, deficiency for GOAT did not affect body weight or fat mass when the mice were fed a standard chow diet. When fed HFD, GOAT-deficient mice had lower body weights but unchanged fat mass (58). Accordingly, transgenic mice with hepatic overexpression of human GOAT and ghrelin had unchange food intake but increased body weight and fat mass and decreased energy expenditure compared with WT littermates fed the same diet rich in medium-chain fatty acids. When switched back to standard chow diet, these differences in body weight, fat mass, and energy expenditure disappeared (58). Overall, lipid oxidation seems to be decreased in transgenic GOAT-ghrelin overexpressors.

In summary, GOAT KO or overexpressor mouse models helped to sketch a completely new physiological role for the ghrelin system: GOAT hereby acts as a nutrient sensor for medium-chain fatty acids and activates ghrelin only when calorie-dense food is available. As a consequence, acylated ghrelin is secreted into the circulation and activates central neuronal circuits CNS about availability, rather than absence, of calories to ultimately facilitate growth, nutrient partitioning and lipid storage.

**Ghrelin and the regulation of glucose metabolism.** The role of ghrelin as a glucose regulating, or as speculated by some, an insulin inhibitor, remains controversial. It has been suggested that ghrelin may have a paracrine or autocrine contribution to the regulation of insulin secretion (29). A logical piece of evidence supporting such a theory is that ghrelin and its receptor are expressed in pancreatic islet cells (29, 33, 124). The controversy, however, originates from whether ghrelin inhibits insulin secretion (19, 23, 98) or enhances insulin secretion (5, 29). Glucose-stimulated insulin secretion is inhibited by ghrelin, as is shown in animal (98) and human (19) studies. Whether ghrelin is capable of directly suppressing insulin secretion to maintain physiological plasma glucose levels remains unclear. It is interesting to note that stomach ghrelin release is suppressed by high glucose levels (121) and may represent a negative feedback mechanism for the maintenance of basal glucose concentrations (23, 29).

Ghrelin has been shown to directly attenuate peripheral insulin action by inhibiting insulin-dependent suppression of gluconeogenesis and insulin-stimulated glycogen synthesis in the liver (77). The mechanism may involve inhibiting insulin signaling by blocking Akt kinase activity and by upregulating gluconeogenic enzymes as demonstrated in hepatoma cell lines (77). Furthermore, ghrelin was shown to influence insulin levels indirectly via the stimulation of glucagon (105), GH (59), cortisol (118), and epinephrine release (78). These four hormones are each known to antagonize insulin action. An additional indirect impact of ghrelin on insulin signaling was demonstrated in cell culture whereby ghrelin treatment reduced gene expression of adiponectin, an adipocyte-derived hormone that enhances insulin sensitivity (87).

The endogenous role of ghrelin in the regulation of glucose homeostasis is even more poorly defined. Ghrelin−/− mice exhibit significantly better glucose metabolism than WT littermates following early exposure to HFD by improving glucose tolerance and lowering plasma concentrations of insulin, glucose, leptin, triglycerides, and cholesterol (128). These data suggest that ghrelin deficiency may protect rodents from HFD-induced hyperglycemia and hyperinsulinemia. Observations from GHSR−/− mouse studies similarly demonstrate a strong trend toward improved insulin sensitivity and glucose tolerance on chow diet (135). When crossing leptin deficient ob/ob mice with ghrelin-deficient mice, Sun et al. (114) found that dKO mice for leptin and ghrelin displayed lower basal glucose and higher insulin concentrations than ob/ob mice while not exhibiting different body fat mass. Both glucose and insulin tolerance tests revealed lower glucose concentrations in ghrelin-deficient ob/ob mice compared with ob/ob mice. In addition, plasma glucose levels normalized during fasting conditions in ghrelin-deficient ob/ob mice. The authors therefore concluded that ghrelin deficiency improved glucose sensitivity and pancreatic β-cell function. Experiments from our own laboratory, however, indicated that glucose disposal is unchanged in ghrelin and GHSR dKO mice, at least when the mice were fed standard chow diet (92). Fed and fasting plasma glucose levels as well as fasting insulin levels were not different, and results from glucose tolerance and insulin tolerance tests failed to differ between genotypes. These findings therefore suggest that ghrelin signaling contributes minimally to glucose homeostasis, at least during prolonged exposure to low-fat diet conditions. In contrast, transgenic mice with neuron-specific (and to a much lesser extent liver-specific) overexpression of ghrelin developed glucose intolerance at 32 weeks of age despite having lower body weights (96).

In conclusion, the effects of ghrelin on insulin secretion and insulin sensitivity have been discussed in a series of in vitro as well as in vivo studies. However, defining ghrelin as a regulator of glucose disposal has been complicated by conflicting reports in the literature. Furthermore, the absence of definitive glucose utilization phenotypes in ghrelin and GHSR dKO and single-KO mice suggests that ghrelin is less relevant to the regulation of glucose homeostasis when mice are fed a chow
diet. Nevertheless, when mice with ghrelin or GHSR deficiency were challenged with HFD, beneficial effects on glucose disposal and insulin sensitivity became apparent.

**Ghrelin, jack of all trades?** In addition to the regulation of energy homeostasis, much ghrelin research had focused on its stimulation of GH secretion. However, recent studies describe a variety of diverse ghrelin functions ranging from effects on growth and development to learning and memory; these are discussed in more detail below.

**Effects of Ghrelin on the GH Axis.** Ghrelin is well known for its ability to stimulate growth hormone-releasing hormone (GHRH) neurons in the hypothalamus through activation of the GHSR1a (7). Nevertheless, both ghrelin-deficient and GHSR-deficient mice have normal growth rates and normal body lengths (113, 115, 127). However, transgenic rats with attenuated GHSR gene expression specifically within the arcuate nucleus of the hypothalamus are smaller than nontransgenic control rats (109). The unaffected growth rates of ghrelin-deficient mice could be explained by the constitutive activity of GHSR in the absence of its ligand (53). However, it remains unclear why GHs deficiency in mice does not affect body weight, whereas rats with hypothalamus-specific GHSR knockdown are smaller than their respective controls. Deficiency for both ghrelin and GHSR leads to a small but significant decrease in body length, which is not observed in either of the single gene knockout mice (92). It is possible that other GH-stimulating signals are able to compensate for the absence of GHSR but only when its signal has been globally disrupted. Hypothalamus-specific GHSR deficiency may not be sufficient to upregulate a compensatory response. Interestingly, loss of GHSR constitutive activity as a result of a missense mutation delays growth but does not affect ghrelin-induced hyperphagia (88, 126). Clearly, the molecular mechanisms utilized by cells to differentiate between ghrelin-dependent and ghrelin-independent GHSR signaling have yet to be defined. Moreover, redundant or compensatory pathways regulating GH-stimulated release have yet to be identified despite evidence supporting that such pathways exist.

**Ghrelin Deficiency Impairs Learning and Memory.** Recent studies demonstrated that ghrelin and its receptor are expressed in regions of the brain other than the hypothalamus, for example the midbrain or the cerebral cortex (25). GHSR is expressed in the hippocampal formation, which is responsible for memory development and spatial learning (47). Circulating ghrelin was shown to enter these areas (34) and was demonstrated to modulate morphological synaptic plasticity (94), which is associated with improved brain function. Thus, it was hypothesized that learning and memory performance would be impaired during states of ghrelin deficiency. Diano et al. (34) showed that ghrelin-deficient mice have a reduced number of spine synapses in the stratum radiatum, a subarea of the hippocampal formation, resulting in an impaired ability to memorize novel objects. Therefore, it seems that ghrelin is capable of translating peripheral metabolic signals into higher brain functions.

**Other Functions of Ghrelin.** Additional functions of ghrelin could be listed, including the regulation of thymopoiesis (35), gastric emptying (31), inflammation and colitis (31), wake/sleep rhythms (117), and aging (111). Peripheral ghrelin was shown to activate dopamine receptors in the ventral tegmental area and dopamine turnover in the nucleus accumbens, thereby increasing food intake in rats (2). Since both brain areas are part of the mesolimbic reward circuitry, peripheral ghrelin has therefore since been implicated in the hedonic control of food intake. Ghrelin administration increased the rewarding value of HFD when administered ad libitum to mice (90). Accordingly, GHSR-null mice displayed lower reward behavior when fed HFD under conditions of dietary restriction. However, it should be noted that homeostatic controls of feeding, e.g., the compensatory hyperphagia after dietary restriction, were not affected by either GHSR deficiency or administration of a ghrelin antagonist to WT mice (90). In summary, recent focus shifts more and more to hedonic mechanisms, such as food reward, to explain the observed orexigenic effect of ghrelin.

**Mutant Mouse Models for PYY**

**PYY and the regulation of food intake and body weight.** Some, but not all, published pharmacological evidence supports a role for PYY in food intake and body weight regulation. However, its physiological contribution to food intake and adiposity regulation remains controversial, as currently available reports about energy metabolism in PYY-deficient mice are conflicting. A recent study showed that PYY-deficient mice develop hyperphagia, leading to a significant increase in body weight and fat mass on a regular chow diet (13). In related studies, acute replacement with PYY3-36 decreased food intake more effectively in PYY-deficient mice than in WT controls, suggesting that PYY3-36 mice are hypersensitive to the anorexigenic effect of PYY. When PYY3-36 was chronically administered for 21 days to both young and old PYY-deficient obese mice or WT controls, body weight and fat mass decreased significantly only in PYY3-36 mice (13). In contrast, Boey et al. (16) found that only female PYY-null mice fed a regular chow diet increased food intake and exhibited modestly increased fat mass whereas males did not. In that study, male PYY-deficient mice differed in neither feeding behavior nor body composition compared with WT littermates on chow diet. When fed an HFD, male PYY-deficient mice did not change their body weight; however, they changed their body composition to increase their relative fat mass compared with WT controls (16). Furthermore, chow-fed male PYY KO mice maintained some resistance to age-induced late-onset obesity. Boey et al. therefore suggested that low PYY levels could predispose rodents to the development of obesity, particularly during aging or conditions of high-fat feeding (16).

Wortley et al. (129) found no effect of PYY deficiency on body weight or body fat in either male or female PYY KO mice on normal chow diet. No effect of PYY deficiency on adiposity was found in male mice irrespective of the diet utilized (129). The only obesity-relevant phenotype in these studies was that female PYY-deficient mice had increased body adiposity after HFD exposure compared with WT littermates. Schonhoff et al. (107) were also unable to recapitulate the suggested proneness to hyperphagia and diet-induced obesity suggested by Boey et al. When fed a chow diet, neither male nor female PYY-deficient mice showed signs of hyperphagia or changes in body weight, fat mass, or hypersensitivity to PYY3-36 administration (107). In summary, there is one report showing a very impressive obesity-relevant phenotype, one that finds a more modest phenomenon, and two publications of independent PYY KO mice that were unable to find any significant body fat or food intake related phenotype.
Ectopic overexpression of PYY in transgenic mice did not change weight gain or food intake when they were fed a chow diet (15). When fed an HFD, PYY transgenic mice did not show decreased body weight but demonstrated a relative reduction in fat mass. Furthermore, food intake between transgenic and WT mice was comparable. Lower fat mass in PYY transgenic mice was attributed to an increase in core body temperature. Boey et al. argue that this phenotype may have resulted from increased expression of thyrotropin-releasing hormone in the paraventricular nucleus of the hypothalamus of PYY transgenic mice. These findings highlight a potentially important role of the hypothalamo-pituitary-thyroid axis in the regulation of thermogenesis in PYY-overexpressing mice (15).

In accordance with increased core body temperature in PYY transgenic mice, PYY has been reported to be associated with energy expenditure in humans (48, 110). One study connects PYY with locomotor activity in mice, which in turn could alter energy expenditure (79). However, the presumptive regulation of energy expenditure by PYY may depend on the dose of PYY used. For example, low doses of PYY delivered intracerebroventricularly (ivc) to WT animals increased locomotor activity (and thereby activity-induced thermogenesis), whereas higher doses induced sedation (79). Importantly, whereas one study reports decreased dark-phase locomotor activity in male PYY-deficient mice (37), another study finds unchanged locomotor activity in PYY-deficient vs. WT mice (129). Therefore, weight gain in PYY-deficient mice may be a function of food intake rather than a result of changes in energy expenditure. This is a relevant consideration when the efficacy of PYY as an antiobesity drug is being assessed. In theory, an ideal antiobesity drug should not only reduce appetite but also increase energy expenditure. Continuing research should therefore elucidate additional energy-regulating mechanisms potentially modified by PYY, such as brown adipose tissue energy metabolism and its characteristic ability to dissipate stored energy as heat.

In summary, loss- and gain-of-function models for PYY exhibit a large variability and indicate either no role in energy balance regulation or a modest to significant role for PYY as an endogenous factor that drives satiety and thermogenesis. There are a number of reasons that may explain such disparate results. First of all, the development of pancreatic polypeptide (PP)-secreting cells can be affected by targeting the PYY gene and therefore has been cedoleted in some of the models reviewed here (107) but not in others (13, 129). Different background strains of the mouse mutants are known to potentially have significant influence on the size of a phenotype. Also, selection of founder pairs can introduce major variability as well as details of experimental design including exact composition of HFDs, light-dark cycles, or single versus group housing, to just name a few.

PYY can bind to a variety of NPY receptors, with comparably highest affinity binding to the NPY2 receptor. Young mice deficient in NPY2 receptor demonstrated a small but significant increase in body weight on regular chow diet; this phenotype was exacerbated in mice fed an HFD. In addition, food intake and fat deposition were also increased in NPY2-deficient mice (81). Edelsbrunner et al. (38) report increased body weight and nocturnal locomotion but decreased diurnal locomotion and feeding in female NPY2-deficient mice fed regular chow diet. Overall food intake in the mice, however, did not change. In contrast, when introduced into the genetic background of leptin-deficient ob/ob mice, NPY2 receptor deficiency markedly attenuated the pronounced adiposity and glucose intolerance (82, 103). Generation of triple-KO mice that are deficient for the Y2 and Y4 receptor as well as for leptin (Y24ob mice) showed reduced body weight and fat mass of the Y24ob mice compared with WT and single-KO littermates (66). Injecting adenovirus-expressing Cre recombinase into the hypothalamus of adult mice with a floxed NPY2 receptor gene, Sainsbury et al. (102) demonstrated that NPY2 receptors are indeed involved in the regulation of energy homeostasis and body weight. Mice with this conditional knockdown of hypothalamic NPY2 receptor had elevated levels of the anorexigenic neuropeptides cocaine- and amphetamine-regulated transcript (CART) and proopiomelanocortin (POMC) as well as increased levels of orexigenic neuropeptides agouti-related peptide (AgRP) and NPY, resulting in decreased body weight and, surprisingly, increased food intake. These effects were transient, disappearing four weeks after the time of virus injection. This evidence further demonstrates the complexity of redundant and compensatory regulatory systems that maintain functional energy homeostasis (102) as this study would suggest that blockade of Y2 receptors in the CNS would be beneficial for the treatment of obesity, whereas PYY3–36 is an agonist that activates Y2 receptors. It appears to be potentially crucial to ensure selective agonism of PYY at only very few Y2 receptors within the mediobasal hypothalamus to possibly achieve weight loss rather than weight gain.

Mutant mouse models were generated to assess the function of additional NPY receptors and are reviewed in more detail elsewhere (69). Briefly, NPY1 receptor-deficient mice had increased body fat mass and hyperinsulinemia but did not change food intake or fasting glucose levels (55, 63, 89). In contrast, NPY4 receptor KO mice had decreased body weight, lower food intake, and lower fat mass (104). NPY2 and NPY4 receptor dKO mice showed a more profound reduction in adiposity, increased food intake, leptinemia, and insulinemia then in mice deficient in either NPY2 or NPY4 receptors alone, suggesting synergism between these receptors in the regulation of energy balance (100). Triple-receptor mutants of NPY1, NPY2, and NPY4 showed a phenotype more similar to that observed in NPY2 and NPY4 receptor double-mutant mice, suggesting that the antiobesity effects of Y2 and Y4 are more relevant than the obesity-promoting effects of NPY1 receptor activation alone (101). Y24ob mice are leaner than the Y2ob and Y4ob KO mice but still obese compared with WT mice (66). Finally, NPY5 receptor deficiency led to the development of mild late-onset obesity, which was characterized by increased body weight and fat mass as well as higher food intake (72).

The individual roles of all NPY receptors in mediating the effects of PYY remain unresolved. For instance, all NPY receptors were shown to bind not only PYY3–36 but also other ligands, including NPY, PP, or PYY1–36. To add even more complexity, a recent report proposes synergistic interactions between Y1 and Y5 receptors (73). In addition, gene deletions for Y1 and Y5 receptors resulted in body weight and adiposity changes that were opposite from those observed in mice deficient in Y2 or Y4 receptors. A more thorough evaluation of the different ligand-receptor interactions vis-à-vis the moieties involved in these interactions and the inhibitory or excitatory events occurring at subreceptor levels may have to be con-
ducted. Furthermore, the central target and key receptor population for a PYY control of energy homeostasis remains unclear. NPY receptor subtypes are expressed in a variety of brain tissues, most notably in the AgRP and POMC neurons of the arcuate nucleus, which were previously shown to be the main centers for the regulation of energy and (mainly hepatic) glucose homeostasis (85). It might be entirely possible that PYY elicits different actions depending on the respective receptor population and the site(s) affected. Moreover, the activity of PYY might be defined by the nutritional and metabolic state of the organism and might depend on a complex differential secretion pattern of the various NPY receptor ligands PYY, PP, or NPY and the input of other neuronal or hormonal signals. To elucidate this complex network, research is needed toward a more site-specific genetic approach, e.g., by generating cell type- or nucleus-specific knockout models or transgenic overexpressors for various NPY receptors. Furthermore, high sequence similarities and overlapping substrate specificities of NPY receptors may facilitate compensatory mechanisms when individual receptors are being knocked out during embryonic development. In this respect, it should be noted that initial studies on mice deficient for NPY itself did not show a significant body weight or food intake (40).

Compensatory mechanisms during development might be responsible for this lack of effect in NPY KO mice. Such compensatory mechanisms, however, seem less present or important in leptin mutant mice, where NPY seems to play a crucial role in developing the full degree of obesity (41). The generation of inducible mouse models would overcome such obstacles and would help to clarify the role of NPY receptors in the regulation of body weight and energy homeostasis via PYY. Importantly, genetic background is an essential and frequently underestimated influence on the phenotype of mouse mutants. As a relevant example, when NPY-deficient mice were backcrossed for seven generations onto a clean C57BL/6 background, they displayed mild obesity and an attenuated fasting-induced refeeding response (108).

In conclusion, some, but not all, studies suggest that PYY functions as a more or less potent anorexigenic signal to reduce food intake, body weight, and body fat mass. It has been reported in one study that PYY−/− mice may be hypersensitive to PYY replacement therapy, ultimately leading to an improvement of obesity. This discovery could be useful for the therapy of obese humans if PYY levels are low in obesity (11, 65). However, not all studies have been able to confirm the proposed reduction of basal PYY levels in obese humans (57, 61, 91). In addition, there is concern that a possible suppressive effect of PYY on food intake may merely be the indirect result of nausea, which was previously described as a major and very frequent side effect of PYY administration in a number of human studies (32, 43, 110). Such side effects would leave only a very small therapeutic window and naturally limit the value and acceptance of PYY3-36 as a treatment for obesity. On the basis of all available data from animal models, PYY may play a relevant role as a physiological regulator of energy balance under certain circumstances. The hope for PYY to succeed as a drug candidate for treatment of human obesity, however, has largely vanished over the recent years, largely as a consequence of massive side effects and disappointing efficacy in human studies. However, important lessons can still be learned about the structure-function relationship of the NPY/PYY/PP peptide family by using increasingly sophisticated combinations of pharmacological tools and genetic gain- or loss-of-function models.

**Effects of PYY on glucose homeostasis.** Effects of PYY on insulin secretion and pancreatic β-cell function are relatively well studied. PYY has no effect on basal glucose, insulin, or glucagon concentrations (18) but dose-dependently inhibits glucose-stimulated insulin secretion (49, 83). Additionally, PYY inhibits carbachol- and arginine-induced glucagon release when injected intravenously (18, 116). Furthermore, PYY infusion can decrease insulin secretion by improving insulin sensitivity, glucose disposal, and HbA1c, a marker of glycosylated hemoglobin (86). In PYY-deficient mice, glucose tolerance did not seem to be overly affected (16). However, in an oral glucose tolerance test, PYY-deficient mice had significantly increased plasma insulin levels (16), which may have been a consequence of altered relative fat mass in that model. The absence of NPY neuron inhibition and activation of POMC neurons following PYY-induced activation of Y2 receptors in the hypothalamus may possibly be an underlying mechanism (12). However, a cross of NPY2 receptor KO mice with leptin-deficient ob/ob mice attenuated the profound hyperinsulinemia and hyperglycemia observed in ob/ob mice (103), suggesting that endogenous activation of Y2 receptors would predominantly drive blood glucose levels up rather than down. In addition, direct inhibition of insulin secretion by PYY through NPY Y1 receptors expressed in pancreatic islets (20), might be possible. Finally, binding of PYY to NPY Y1 and NPY Y2 receptors in the brain stem could modulate vagal output, directly affecting pancreatic insulin secretion (52, 71).

**Additional functions of PYY: from gut motility to bone morphology.** Most effects of PYY are inhibitory, such as the inhibition of gastric, pancreatic, and intestinal secretion (6, 119) or reduced gastrointestinal motility and gastric and gallbladder emptying (6, 62). However, a recent study in PYY-deficient mice with surgical small bowel resection and exclusive enteral nutrition suggests that PYY administration may protect intestinal function, e.g., by increasing mucosal weight and crypt cell proliferation, crypt depth, and villus height (133). In addition, it was discovered that PYY may also play an important role in the regulation of bone metabolism: PYY−/− mice exhibit reduced bone turnover and eventually develop an osteopenic phenotype (129). Despite having increased body mass, PYY−/− mice have significantly reduced bone density compared with WT mice (129). Interestingly, adenoviral knockdown of NPY2 receptors in the hypothalamus of adult mice induced a twofold increase in trabecular bone volume as well as greater trabecular number and thickness (9). This surprising finding highlights a central control mechanism of bone formation and may be explained by the central action of the gut hormone PYY. However, it remains largely unclear whether endogenous PYY circulating in blood is physiologically relevant, either directly or indirectly, for intrinsic pathways controlling osteogenic remodeling.

**Conclusion**

Targeted mouse mutagenesis efforts have generated a series of mouse mutants with relevance for the ghrelin-GHSR and the PYY-NPY receptor pathways. These mouse models provide useful tools for studying the endogenous contribution of these impor-
tant gut hormones to the regulation of energy and glucose homeostasis. Even though a great amount of knowledge has been gained that way regarding the physiological function of these gut hormones in metabolic control, many questions still remain unanswered. For example, the central versus peripheral role of ghrelin in mediating changes in energy efficiency and fuel preference and the inhibitory effects of PYY3–36 on food intake and body weight gain still appear to depend on an unclear set of specific environmental or genetic conditions and are lacking a clear mechanistic basis. To date, mouse mutagenesis approaches have focused on global gene deletions or viral promoter-driven transgene expression. Ongoing efforts are using germline mutations to achieve cell- and tissue-specific gene modification. Such a strategy will allow delineating the specific effects of these gut peptides on each of their different target tissues. Subtle differences between global and hypothalamic GHSCR deficiency, for instance, facilitated the differentiation of the divergent effects of ghrelin signaling on growth versus energy homeostasis. The mouse models described thus far, despite having shortcomings related to potential early adaptations during embryonic development, nevertheless revealed the complex physiological functions of ghrelin and PYY and corrected simple models deduced from less physiologically relevant pharmacology studies. Importantly, the use of knockout mouse models has led to the discovery of novel functions of ghrelin and PYY, including roles in memory and learning or a participation in bone remodeling. The use of existing and more refined future mouse mutants will therefore remain an essential tool for the study of both the ghrelin and the PYY pathways.

DISCLOSURES
No conflicts of interest are reported by the author(s).

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