Neuropeptide Y and sex hormone interactions in humoral and neuronal regulation of bone and fat

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The hypothalamus regulates the skeleton and adipose tissue via endocrine mechanisms. Changes in sex steroid levels in menopause and aging are central to the associated changes in bone mass and adiposity. Whereas many of these effects occur via direct actions on osteoblasts or adipocytes, sex hormones can also mediate effects on bone and adipose tissue via interaction with neuronal pathways. A key hypothalamic regulator of bone and adipose tissue is neuropeptide Y (NPY), which coordinately influences these tissues via effects on neuroendocrine and sympathetic nervous output. Better understanding of the interaction between NPY and sex steroids in regulating skeletal and energy homeostasis could lead to more effective treatments for osteoporosis and obesity.

Neuroendocrine control of bone and fat

Osteoporosis and excess central adiposity, increasingly prevalent in ageing populations, increase the risk of fractures and diseases such as type-2 diabetes mellitus, and adversely affect both quality of life and survival. The regulation of bone and adipose tissue mass has traditionally been studied independently, but osteoblasts and adipocytes are derived from the same mesenchymal precursor cells, and recent findings that the bone- or fat-derived hormones, osteocalcin and leptin, have significant effects on energy homeostasis and bone [1], respectively, now suggest that bone and adipose tissue share common regulatory pathways. The hypothalamus acts as a pivotal regulator of homeostasis in bone and adipose tissue via regulation of hypothalamo-pituitary axes. For instance, activation of the hypothalamo-pituitary-adrenal axis, or inhibition of the hypothalamo-pituitary-somatotropic or -gonadotropic axes (as in stress or negative energy balance), can affect circulating concentrations of cortisol, insulin like growth factor-1 (IGF-1) and sex steroids that inhibit bone formation while promoting conservation of fat mass, particularly central adiposity 2, 3 and 4. Sex hormones – a major focus of this review – influence bone and fat via direct actions on osteoblasts or adipocytes 2, 3, 5, 6, 7 and 8. Recently, however, it has been recognized that sex hormones can influence these tissues via the neuropeptide Y (NPY) system (Box 1) 9, 10, 11 and 12, that also regulates bone and adipocyte homeostasis via actions in the hypothalamus and peripheral tissues 9, 13 and 14. In this review we examine the role of sex hormones in skeletal and energy homeostasis, with particular focus on their interaction with NPY. Whereas research into the isolated effects of sex hormones or the NPY system on bone and adipose tissue homeostasis has led to identification of potential novel drug targets for the treatment of osteoporosis or central obesity, emerging research into the interaction of these systems could pave the way for even better treatments for these conditions, particularly in the context of reduced circulating concentrations of sex hormones, as in menopause or ageing in women and in men.
Effects of sex hormones on bone and adipocyte homeostasis – direct actions

Sex hormones are well known for their regulation of sexual development and reproductive functions 15 and 16. The most potent and dominant form of estrogen is 17β-estradiol. Estrogen mediates its actions by binding to the nuclear estrogen receptors alpha and beta (ERα and ERβ). ERα is primarily expressed in the uterus, liver, kidneys and heart, whereas ERβ is mainly expressed in the ovaries, the hematopoietic system and in the CNS [17]. Androgens are C-19 steroids secreted mainly from the testes and adrenal glands. The main gonadal androgen in males is testosterone, bound in the circulation to albumin and sex hormone-binding globulin [8]. Testosterone can be irreversibly converted in peripheral tissues via the enzyme 5α-reductase to the more potent 5α-dihydrotestosterone (DHT), and both DHT and testosterone can activate androgen receptor (AR) 16 and 18. Furthermore, testosterone can also be converted to 17β-estradiol by an enzyme complex known as aromatase [16]. In addition to effects on reproductive organs and tissues, sex hormones play an essential role in bone metabolism.

The expression of both ERα and ERβ by bone cells opens the possibility of direct estrogen signaling in this tissue. Indeed, sex steroids such as estrogen indirectly regulate osteoclast action via signaling in the osteoblast [8], and sex hormone deficiency results in bone loss characterized by increased bone resorption [7]. Furthermore, the loss of estrogen following ovariectomy in rodents suppresses bone formation [19], whereas 17β-estradiol increases bone formation in vivo [20], and independently of changes in bone resorption [21]. Estrogen inhibits osteoclastogenesis by suppressing the production of the receptor activator of NF-κB ligand (RANKL), thereby increasing the production of the soluble decoy receptor osteoprotegerin (OPG) 22 and 23, as well as via regulation of various bone-acting cytokines such as interleukins IL-1 and IL-6 and tumor necrosis factor (TNF) 24 and 25. Moreover, estrogen (as well as testosterone) inhibits bone resorption 26 and 27 by inducing osteoclast apoptosis [28] and suppressing activity of mature osteoclasts through direct receptor interactions [29]. In brief, estrogen has numerous effects on bone – some via direct tissue actions – to stimulate bone formation and decrease bone
resorption.

In addition to estrogen effects on bone, androgens are important regulators of bone cell activity and bone mass in both humans and mice [30]. ARs are found in both osteoblasts and osteoclasts; however, they are primarily expressed in osteoblasts and to a greater degree in cortical than cancellous bone [31]. The presence of AR in bone cells suggests that androgens influence bone via direct tissue effects. Indeed, selective deletion of AR in mature osteoblasts decreases bone mass [6] and reduces bone formation [7], whereas chronic subcutaneous testosterone treatment in rats stimulates bone formation 5 and 32. These effects of androgens on bone mass and bone formation are consistent with the fact that low serum testosterone levels are a risk factor for fracture in men, nearly doubling the risk of hip fracture in men over 60 years of age [33]. Interestingly, androgens also seem to be important regulators of bone mass in women, because women with androgen-insensitivity syndrome have lower bone mineral density despite estrogen and progesterone treatment [34]. In summary, androgens influence bone mass in men and likely also in women, and this can occur via direct effects on bone cells, particularly osteoblasts. This is in keeping with the finding that failure of osteoblastic function in the early postmenopausal period in females, as well as in aging males, is crucial for the development of osteoporosis 4 and 33.

In addition to effects on bone homeostasis, sex hormones have pronounced effects on adipose tissue. Indeed, estrogen deficiency in female animals is associated with hyperphagia and increased body weight and adiposity, particularly visceral adiposity 2, 3, 35 and 36. In women, some studies (although not all) report that menopause is associated with weight gain, even after controlling for the effects of age 37 and 38. Discrepancies among these studies are probably due to the fact that body weight – often used in large epidemiological studies – is not a reliable index of body composition, and more accurate estimates of body composition and fat distribution would lead to more consistent findings. In fact, when central fat is specifically measured, the majority of studies observe increased abdominal or visceral adiposity at menopause 37 and 38. In both ovariectomized animals and estrogen-deficient women, administration of exogenous estrogens reduces body weight gain, prevents abdominal or visceral fat gain and reduces loss of fat-free mass and muscle strength 3, 39 and 40, demonstrating the pivotal role of estrogens in regulating body weight and body composition. ERα and ERβ receptors are present on adipocytes, and some of the effects of estrogens on fat mass take place via direct actions on adipocytes to stimulate lipogenesis and lipid oxidation, as recently reviewed [41], with regulation via genomic and non-genomic regulation pathways [42], albeit other effects of estrogen on fat mass are likely to be mediated within the CNS (reviewed in Ref. [43]).

Epidemiological studies have demonstrated that serum testosterone levels are inversely associated with whole body and regional fat mass 44 and 45, suggesting a role for androgens in adipose tissue metabolism. In men, the observed gradual decline in circulating androgen levels with aging is accompanied by increased total and abdominal fat [46]. Indeed, administration of aromatizable androgens such as testosterone in older men reduces both total and abdominal fat and increases lean mass 47, 48 and 49, whereas in vivo and in vitro studies show that androgens play a significant role in altering fat accumulation and distribution 50, 51, 52 and 53. ARs are found in adipose tissue, with more being present in visceral compared to subcutaneous depots [54]. This is consistent with the greater influence of androgen action in visceral adipose tissue. Via AR, androgen exerts important functions on adipose tissue metabolism such as lipolysis and lipogenesis, preadipocyte proliferation and adipogenesis (reviewed in Ref. [55]). Thus, reduction in androgen levels with ageing [37] could contribute to increased adiposity – particularly central adiposity [56] – that has been observed in older males, and this could be mediated at least in part via direct androgen signaling on adipocytes.

Taken together, these findings show that sex steroids represent an important regulatory influence in skeletal and adipose tissue. Local ER and AR expression on bone and adipose cells reinforces the crucial role of direct signaling in these tissues. Sex steroids, however, also interact with other regulatory systems such as the NPY system to mediate their effects on bone and adipose tissue. Before reviewing the recent data showing the interaction of sex hormones and NPY in the regulation of bone mass and adiposity, we will summarize the effects of the NPY system per se.
Hypothalamic actions of the NPY system in bone and adipose tissue

Increasing evidence has linked efferent neural signaling from the hypothalamus to bone remodeling. Following initial studies on the central effects of leptin to reduce bone mass via activation of the sympathetic nervous system [57], a number of neuromodulatory molecules have been shown to influence the function of osteoblasts and osteoclasts 58 and 59. One of the most powerful centrally-mediated skeletal effects described to date involves the NPY system. Site-specific overexpression of NPY in the hypothalamus of mice markedly reduced the capacity of osteoblasts to produce bone 60 and 61. The unilateral injection used in these studies excluded the potential for effects resulting from damage of the arcuate nucleus, as evident in studies using monosodium glutamate [62]. In mice overexpressing NPY in the hypothalamus, the rate of mineralized and unmineralized [61] bone formation was markedly (up to 7-fold) reduced on both the cortical and cancellous envelopes, resulting in a significant loss of bone mass. This loss of bone took place despite increasing body weight [61], a powerful stimulus for the accrual of bone mass [63].

Consistent with the central inhibitory effect of NPY on osteoblast activity being mediated by Y2 receptors, conditional deletion of Y2 receptors from the hypothalamus of adult mice produced a generalized increase in osteoblast mineral apposition rate (MAR), with no evidence of changes in the bone resorption indices of osteoclast surface or osteoclast number [9]. Remarkably, MAR in cancellous bone of hypothalamus-specific Y2−/− mice was elevated 2-fold, in parallel with similar changes in cancellous bone volume [9]. In contrast to the central NPY overexpression model, that exhibited not only marked increases in body weight and adiposity but also hormonal changes (that could have contributed to the associated bone loss – e.g. increased serum leptin [57] and corticosterone [64] concentrations with concomitant reduction in serum IGF-1 levels 57 and 65), central deletion of Y2 receptors did not produce any sustained changes in body weight or adiposity; serum leptin, IGF-1, T4 and calcium concentrations were also unaltered when measured in the non-fasted state [9]. Whereas serum corticosterone concentrations were elevated in these mice [9], this would be expected to reduce bone formation [64] rather than contribute to the observed increase in bone mass. These findings indicate that the bone anabolic phenotype observed in hypothalamic Y2-receptor-deficient mice could be mediated by non-humoral factors, such as alterations in sympathetic nervous output to bone, an area of research in which studies are now underway.

A recent publication confirmed the inhibitory action of NPY on osteoblast activity, where NPY−/− mice showed an increase in cortical and cancellous bone mass in both axial and appendicular regions of the skeleton [61]. In cancellous bone, loss of NPY signaling induced an increase in osteoblastic MAR similar to that evident in hypothalamus-specific Y2−/− mice, with an additional increase in the surface extent of bone formation [61]. These osteoblast changes were associated with doubling of the rate at which cancellous bone is formed in these mice, with a concomitant increase in bone volume. In cortical bone, cross-section area and thickness were significantly greater in NPY−/− mice, and this effect was also due to increased bone formation [61]. Whereas these findings are in marked contrast to an initial assessment of vertebral cancellous bone in NPY−/− mice [57], further work by the same group showed significant increases in bone mass following loss of NPY-producing neurons from the arcuate neurons of the hypothalamus after monosodium glutamate treatment [62]. Thus, a number of lines of evidence now indicate that NPY acts within the hypothalamus to reduce bone mass, most likely via inhibition of osteoblast activity.

The positioning of NPY as a regulator of bone could offer a means by which the hypothalamus coordinates the relationship between bone mass and adiposity. It is particularly interesting that the relationship between hypothalamic NPY signaling and osteoblast activity is consistent with the relationship between body weight and bone mass. Hypothalamic NPY expression is highest during times of insufficient energy intake (Box 1). Elevated hypothalamic NPY expression, in addition to stimulating food intake and reducing physical activity and energy expenditure to protect against excessive body weight loss (Box 1), also inhibits bone formation [61]. This repression of osteoblast activity in energy deficit would have several benefits – conserving energy for essential processes and increasing the availability of minerals stored in bone, while not placing weight-bearing at risk because body weight is lower in periods of energy deficiency. In
contrast, during periods of energy excess in rodents, hypothalamic NPY expression is decreased, at least in the initial months [66], and this reduction of hypothalamic NPY expression could conceivably stimulate osteoblast activity, thereby increasing bone mass at a time when weight-bearing due to increased adiposity is higher. In this manner, the NPY system is ideally placed to monitor body weight and modulate bone mass accordingly. This relationship has the potential to offer weight-bearing-like responses independent of actual bone loading, that is of potential clinical benefit for the maintenance of bone mass in patients on long-term bed rest. This dual regulation of bone mass and adiposity by NPY also has clinical implications in managing obesity. As lifestyle weight loss interventions in humans induce physiological changes that promote weight regain that could be mediated by increased hypothalamic NPY-ergic tonus (recently reviewed in Ref. [67]), it is conceivable that any such increase in central NPY expression could contribute to bone loss that might not necessarily be recovered by the time weight is regained, with possible detrimental effects for subsequent fracture risk. This interesting area of research requires further work to elucidate more clearly the bone response to weight loss.

**Local actions of the NPY system on osteoblasts and adipocytes**

Whereas NPY signaling in the hypothalamus regulates the activity of osteoblasts in the periphery, there appears to be other, non-central actions of the NPY system on bone formation. NPY<sup>−/−</sup> mice, in which NPY was specifically replaced only in the hypothalamus via virus-mediated transgene expression, showed only 50% normalization of the increased rate of mineral apposition (MAR) observed in germline knockout mice [61]. This partial effect highlights the potential for NPY actions in other areas in addition to its established function in the hypothalamus. Consistent with non-central effects of the NPY system on bone, the Y1 receptor has been identified as mediating peripheral actions of the NPY system on bone. Analysis of Y<sup>1−/−</sup> mice revealed an elevated bone-mass phenotype in association with a generalized increase in bone formation on both cancellous and cortical surfaces [14], similar to the phenotype of Y<sup>2−/−</sup> mice [9]. In contrast to the hypothalamus-specific action of Y2 receptors on bone, however, deletion of Y1 receptors from the hypothalamus had no effect on bone cell activity or bone volume, indicating that the effects of Y1 receptors on bone metabolism are mediated by non-hypothalamic receptors [14]. It is possible that Y1 receptors in regions of the brain other than the hypothalamus could be involved in mediating actions of NPY on bone; however, a direct effect of the NPY system on osteoblastic cells acting via locally-expressed Y1 receptors is more likely. Indeed, Y1 expression has been identified in mature osteoblasts and osteocytes, with expression levels increasing with greater differentiation [68]. This is supported by in situ hybridization that revealed Y1 receptor expression in osteoblasts on endocortical and cancellous surfaces within femoral bone tissue [69]. Moreover, these cells produce NPY and respond to mechanical load by reducing NPY expression [68], consistent with local regulatory action on osteoblastic activity. While hypothalamic NPY, acting through Y2 receptors, is ideally placed to modify bone mass in a generalized manner throughout the skeleton, NPY produced in bone itself and acting on Y1 receptors could play an important role in regulating bone mass, enabling local modulation of the generalized central signals. The load-responsive nature of osteoblastic NPY expression is particularly appropriate for ensuring that a generalized bone anabolic effect is applied in an appropriate spatial manner for maximum weight-bearing effect. This latter circuit offers an attractive target for pharmacological modulation and opens the possibility of increasing bone mass without affecting central NPY-ergic pathways. Investigation of mice with osteoblast-specific Y1 receptor deletion, currently under development in our laboratory, will be able to test these hypotheses. These effects of the NPY system on bone via Y1 and Y2 receptors are summarized in Figure 1.

Besides having direct actions influencing bone, the NPY system can exert direct effects on white adipose tissue. A high-fat diet combined with elevated glucocorticoid levels due to stress stimulates the production and release of NPY in peripheral sympathetic neurons as well as in adipose tissue [11]. This increased peptide level coincides with increased Y2 receptor expression in adipocytes, and blocking Y2 receptors specifically in adipocytes abrogates the increase in fat mass [11]. Therefore, while action of the satiety hormone peptide YY (PYY) on Y2 receptors in the brain would tend to reduce adiposity via reductions in food intake and metabolic rate (Box 1), agonism of Y2 receptors on adipocytes appears to have opposite effects, and instead promotes fat accumulation, at least under conditions of a high-fat diet and stress [11]. In light of the finding...
that deletion of hypothalamic Y2 receptors increases bone mass, hypothalamic rather than peripheral Y2 receptors could be involved in matching an appropriate bone mass with body weight. Adipocytes also appear to undergo direct regulation via Y1 receptors that are expressed on adipocytes and preadipocytes. Here, Y1 receptors promote preadipocyte proliferation in response to NPY 70 and 71, mediate the anti-lipolytic effects of NPY, and inhibit lipolysis in rodent and human adipocytes in vitro 70 and 72. Therefore, agonism of Y1 receptors in the hypothalamus (Box 1) as well as in adipocytes 71, can induce changes that would promote fat accretion and weight gain, whereas Y1 receptor agonism would be expected to inhibit bone formation via direct effects on bone, judging from the high bone mass of Y1 receptor knockouts. As such, hypothalamic Y2 rather than Y1 receptors are more likely to be involved in the coordinated regulation of bone and fat mass. The role of Y1 and Y2 receptors in local regulation of adipocyte function is summarized in Figure 1.

![Figure 1](image)

**Figure 1.** Sex-steroid interactions with NPY in the regulation of bone and fat. Negative energy balance, such as during starvation, enhances NPY expression in the hypothalamus, which in turn inhibits osteoblast activity and anabolic bone formation while promoting regain of lost fat mass. This process involves agonism of central Y2 receptors and neuronal efferent signaling to the periphery. (a) In osteoblasts, NPY released from sympathetic nerve terminals or potentially from osteoblasts (that respond to mechanical loading with a decrease in NPY expression) also signals via Y1 receptors to inhibit osteoblast activity. (b) In adipocytes, NPY from sympathetic nerves or produced locally can signal via Y1 and Y2 receptors to promote adipogenesis and fat accumulation. Sex steroids interact with this pathway at multiple locations. Estrogen deficiency (green) stimulates hypothalamic NPY production, thereby indirectly repressing osteoblast activity and increasing adipocyte activity. Testosterone (red) appears to be required for the stimulatory effect of Y1 deletion on osteoblast activity.

**Actions of other members of the NPY system in the control of bone**

The complexity of the NPY system (Box 1) suggests the potential for multiple regulatory influences on bone. While pancreatic polypeptide (PP) appears to have no effect in bone, and
PP-overexpressing transgenic mice have a similar bone mass to wild-type mice [73], reports concerning PYY are conflicting. In fact, PYY−/− mice have an osteopenic phenotype [74]. This finding is inconsistent with reports correlating elevated serum PYY levels with reduced bone mass in patients with anorexia nervosa [75]. Moreover, the osteopenic phenotype of PYY−/− mice is in contrast to the high bone mass of NPY−/− mice [60], despite PYY and NPY both able to signal through the same receptors [76]. Potentially, a mouse model that allows conditional overexpression of PYY in osteoblasts will shed light on the specific role of PYY in skeletal homeostasis.

To date, only Y1, Y2 and Y4 receptors have been studied with respect to effects on bone. In contrast to the situation with the Y1 and Y2 receptors, germline deletion of Y4 receptors did not induce changes in bone structure or cellular activity [73]. Furthermore, Y4 is predominantly activated by the ligand PP [77], that when overexpressed also had no skeletal effects [73]. However, Y4 receptors could play a modulatory role in bone in concert with Y2 receptors. Mice lacking both Y2 and Y4 receptors displayed a synergistic (3-fold) increase in cancellous bone volume and osteoblast activity compared to Y2−/− mice [73]. By contrast, cortical bone mass was significantly reduced in these animals compared to wild type. The increase in bone volume in Y2−/−Y4−/− mice was coincident with a decrease in adiposity and a marked decrease in serum leptin [73]. Leptin deficiency increases cancellous and decreases cortical bone mass via a mechanism separate to that involved in Y2−/− mice 9 and 57. The added increase in cancellous bone volume and decrease in cortical bone mass in Y2−/−Y4−/− mice could therefore represent the additive effects of deficiencies in both the leptin and Y2 pathways to bone, further reinforcing the separate nature of the two pathways and the complexity of neural inputs to the osteoblast.

Taken together, these findings demonstrate that NPY strongly regulates bone mass through both Y2 and Y1 receptor signaling, with deletion of Y2 receptors resulting in greater bone formation via effects in the hypothalamus, and deletion of Y1 receptors having a similar effect via non-hypothalamic pathways, most likely acting directly on bone cells (Figure 1). The powerful pro-osteoblastic signals generated by inhibition of the NPY pathway to bone offer potential therapeutic application in common osteoporosis, such as post-menopause and with aging. While anti-resorptive agents are numerous, bone anabolic agents have proved difficult to generate, and only a single option is currently available [78]. The need for development of new anabolic treatments is clear. Moreover, the association of osteoporosis with reduced sex-steroid production warrants an understanding of how these pathways interact under hypogonadal conditions.

**Interactions between sex hormones and the NPY system in the control of bone and fat**

As outlined, the expression of ER and AR on bone cells and the characteristic in vitro changes subsequent to sex-steroid treatment have reinforced the role of direct action of these hormones on bone metabolism. However, indirect actions are also evident. Namely, a recent study demonstrated that estrogen signaling in the brain contributes to the regulation of the skeleton, with intracerebroventricular estrogen replacement in mice providing at least 1/3 of the total cancellous bone formation stimulated by estrogen supplementation [79]. Part of this regulation could conceivably take place via modulation of central NPY expression. Indeed, estrogen deficiency transiently increases hypothalamic NPY expression [80]; this could contribute to the bone loss (by suppressing osteoblast activity) and fat gain associated with estrogen deficiency because hypothalamic NPY overexpression reduces bone mass and increases adiposity 60 and 65. Consistent with this mechanism, mean wall thickness, a direct measure of osteoblast activity, is reduced following ovariectomy [19]. Moreover, estrogen replacement after ovariectomy in rodents not only normalizes the elevated hypothalamic NPY expression, but also attenuates ovariectomy-induced bone loss and fat accumulation. These interactions between estrogen and NPY in the regulation of osteoblasts and adipocytes are shown in Figure 1. Further insight into the role of NPY in mediating bone loss and fat gain in response to sex steroid deficiency is currently under investigation in our laboratory, with investigation of gonadectomy in male and particularly female NPY−/− mice.

As shown above, lack of Y2 receptor signaling by members of the NPY family such as NPY results in elevated bone formation and bone mass [9]. Interestingly, while gonadectomy induces a significant reduction in cancellous bone volume in both wild-type and Y2−/− mice [13],...
cancellous bone volume in gonadectomized $Y_2^{-/-}$ mice remains significantly greater than that in wild-type mice [13]. Importantly, the elevated osteoblast activity that characterizes the skeletal phenotype of $Y_2^{-/-}$ mice was maintained following gonadectomy in both female and male mice [13]. Moreover, protection against gonadectomy-induced bone loss was also evident following hypothalamus-specific deletion of Y2 receptors in both male and female mice [13]. This effect is attributable to improved bone formation, and is evident despite coincident increases in bone resorption [13]. Interestingly, the increase in fat mass that typically occurs after ovariectomy is prevented by germline deletion of Y2 receptors, and in male mice body weight and fat mass were consistently lower than wild type regardless of sex hormone status. This indicates a role for Y2 receptors in the accumulation of adipose tissue in the hypogonadal state, and demonstrates that hypothalamic Y2 receptors constitutively restrain osteoblast activity even in the absence of sex hormones [13]. Thus, inhibition of hypothalamic NPY signaling via Y2 deletion provides relative protection against ovariectomy-induced bone loss and deterioration of bone microarchitecture in long bones, and inhibition of hypothalamic Y2 activity as a therapy for estrogen-deficient bone loss could thus be particularly effective.

Despite similarities in the cancellous and osteoblastic responses to Y1 and Y2 receptor deletion, important differences in the effects of sex-steroid deficiency are apparent in these models. In female $Y_1^{-/-}$ mice, ovariectomy-induced osteopenia was reduced, attributable to the elevation in MAR, compared to that seen in wild-type mice [36]. Increased bone resorption resulting from sex steroid deficiency induces a subsequent increase in bone formation, albeit insufficient to prevent loss of bone. In wild-type and $Y_1^{-/-}$ mice however, the relative increase in bone formation between sham-operated and ovariectomized mice is only half that evident in $Y_2^{-/-}$ mice [36]. In male $Y_1^{-/-}$ mice, a unique relationship was evident between NPY signaling and orchidectomy. The characteristic increase in MAR seen in these animals was abolished by androgen deficiency [36]. As a result, bones from orchidectomized $Y_1^{-/-}$ mice were indistinguishable from similarly operated wild-type mice. This finding suggests that the bone anabolic responses mediated by Y1 deletion depends on intact androgen signaling (Figure 1).

Thus, in the osteoblast, the presence of androgens is likely necessary for the anabolic activity of the Y1-deficient pathway. This study suggests that in addition to hypothalamic Y2 receptors modulating estrogen effects, osteoblastic Y1 receptors represent a novel site of interaction between classic endocrine and neural pathways in the regulation of bone homeostasis. Reduced androgen levels are a known risk factor for fracture in men [33] and are associated with reduced bone mass in women [81], and it could be that these relationships are in part the result of loss of Y1-mediated effects in bone, perhaps associated with loss of response to mechanical loading [68]. Moreover, any putative treatment regime aimed at attenuating Y1 activity to stimulate osteoblastic activity would benefit from sufficient endogenous androgens or low-dose adjunctive androgen treatment.

Concluding remarks

In summary, the hypothalamus has long been appreciated to be fundamental to the control and coordination of peripheral homeostatic activity. Historically, this has been viewed in terms of the extensive neuroendocrine system resulting from processing of hypothalamic signals relayed to the pituitary. Through these actions, endocrine signals are integrated throughout the body, modulating a vast array of physiological processes. Importantly, our understanding of the responses to these endocrine signals is crucial for the diagnosis and management of many disease processes. Sex steroids are a prime example of the power of endocrine signaling, with fundamental regulatory control over the production and maintenance of not only reproductive tissues, but also that of skeletal and adipose tissue. More recently, neural processes emanating from the hypothalamus are increasingly appreciated as powerful modulators of peripheral tissues. The NPY system is a clear example of this, exerting significant control over the functions of osteoblasts and adipocytes via effects in the hypothalamus and within bone and fat. Moreover, far from being distinct processes, neuroendocrine and neuronal pathways from the hypothalamus appear to act as a single coordinated regulatory system far more subtle and complex than when each is viewed in isolation. In this manner, hypothalamic regulation can now be viewed as a summation of both neuroendocrine and neural influences. As a result, our existing endocrine-based understanding of diseases such as osteoporosis and obesity could be improved following
integration of neural inputs into the process. Nevertheless, our overall understanding of the NPY system remains incomplete; Box 2 presents a series of outstanding questions that will need to be answered before effective NPY-related therapeutics can be developed for these important human conditions.

**Box 2. Outstanding Questions**

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<th>General Questions</th>
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