Review

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

Ayse Zengin[1], Lei Zhang[2], Herbert Herzog[2,3], Paul A. Baldock[1,3*] and Amanda Sainsbury[2,4*]

[1] Osteoporosis and Bone Biology Program, Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, Sydney, New South Wales (NSW), Australia
[2] Neuroscience Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, Sydney, NSW, Australia
[3] Faculty of Medicine, University of NSW, Sydney, NSW, Australia
[4] School of Medical Sciences, University of NSW, Sydney, NSW, Australia

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 [2–4]. Sex hormones – a major focus of this review – influence bone and fat via direct actions on osteoblasts or adipocytes [2,3,5–8]. Recently, however, it has been recognized that sex hormones can influence these tissues via the neuropeptide Y (NPY) system (Box 1) [9–12], that also regulates bone and adipocyte homeostasis via actions in the hypothalamus and peripheral tissues [9,13,14]. 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 [15,16]. 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 [16,18]. 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) [16,18]. 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.

Corresponding author: Herzog, H. (h.herzog@garvan.org.au)
* These authors contributed equally to the manuscript..

  doi:10.1016/j.tem.2010.02.004

Box 1. The NPY system

The NPY system consists of three 36-amino acid peptides – NPY, peptide YY (PYY) and pancreatic polypeptide (PP) – that act on at least five G protein-coupled receptors (Y1, Y2, Y4, Y5 and y6) to mediate numerous physiological functions [76,82,83]. NPY is a neuromodulator predominantly expressed in the nervous system, notably in the hypothalamus [84,85] and is co-localized with catecholamines in sympathetic nerve terminals where it is thought to regulate sympathetic output [86,87]. On the other hand, PYY and PP are released into the circulation from gut tissues, notably L cells in the colon [88] and F cells in the pancreatic islets of Langerhans [89], respectively. Animal studies show that hypothalamic expression and secretion of NPY is markedly increased by energy restriction, and this contributes to adaptive responses such as increased appetite, reduced physical activity and reduced metabolic rate that tend to promote weight regain [67]. Human evidence that central NPY-ergic activity could be elevated in negative energy-balance comes from the finding that anorexia nervosa is associated with increased concentrations of NPY in cerebrospinal fluid [67]. The obesogenic effects of NPY are thought to be largely mediated within the hypothalamus, particularly via agonism of Y1 and Y5 receptors [90]. By contrast, PYY and PP are released into the circulation in response to food ingestion and are known to reduce food intake and stimulate metabolic rate, predominantly via agonism of Y2 and Y4 receptors, respectively, in the brain stem and hypothalamus [91–97]. In addition to effects in the brain, the NPY system regulates energy homeostasis via actions on Y1 and Y2 receptors in peripheral tissues such as the liver and adipocytes [11,98]. Besides effects on energy balance, the NPY system regulates numerous processes such as fluid balance [99], immune function [100], circadian rhythms [101,102], growth [103,104], reproduction [105,106] and skeletal function.

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) [22,23], as well as via regulation of various bone-acting cytokines such as interleukins IL-1 and IL-6 and tumor necrosis factor (TNF) [24,25]. Moreover, estrogen (as well as testosterone) inhibits bone resorption [26,27] 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 [5,32]. 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 [4,33].

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 [2,3,35,36]. In women, some studies (although not all) report that menopause is associated with weight gain, even after controlling for the effects of age [37,38]. 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 [37,38]. 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 [3,39,40], 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 [44,45], 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 [47–49], whereas *in vivo* and *in vitro* studies show that androgens play a significant role in altering fat accumulation and distribution [50–53]. 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 [58,59]. 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 [60,61]. 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 [57,65]), 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 coordinately regulates 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. $NPY^{-/-}$ 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 $Y1^{-/-}$ 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 $Y2^{-/-}$ 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 affect 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

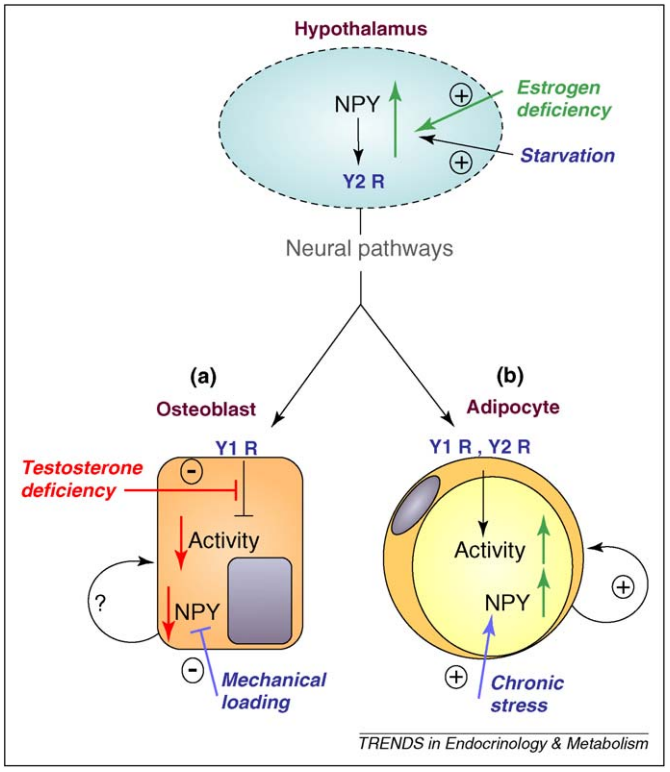

Figure 1. Sex-steroid interactions with NPY in the regulation of bone and fat. Negative energy balance, such as during starvation, enhances NPY expression in the hypothalamus, that in turn inhibits osteoblast activity and bone formation while promoting regain of lost fat mass. This process involves agonism 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.

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 [70,71], mediate

the anti-lipolytic effects of NPY, and inhibit lipolysis in rodent and human adipocytes *in vitro* [70,72]. 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.

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 [9,57]. 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 [60,65]. 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 $Y2^{-/-}$ mice remains significantly greater than that in wild-type mice [13]. Importantly, the elevated osteoblast activity that characterizes the skeletal phenotype of $Y2^{-/-}$ 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 $Y1^{-/-}$ 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 $Y1^{-/-}$ mice however, the relative increase in bone formation between sham-operated and ovariectomized mice is only half that evident in $Y2^{-/-}$ mice [36]. In male $Y1^{-/-}$ 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 $Y1^{-/-}$ 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 conditionsä.

Box 2. Outstanding Questions

General Questions

- What impact do these NPY-mediated pathways have on human health and disease?
- Can understanding the interaction of endocrine and neural pathways improve diagnosis and/or treatment decisions in diseases involving altered bone and fat metabolism?
- Do the pleiotropic actions of the NPY system preclude it as an effective therapeutic agent? Can we specifically target peripheral actions?
- What roles do other components of the NPY system (PYY, PP) play in bone and fat metabolism?

NPY bone-related issues

- What are the functions and mechanisms of peripherally expressed NPY in the osteoblast/adipocyte?
- What is the role of the sympathetic nervous system in mediating the bone anabolic response following loss of hypothalamic Y2 receptor signaling?

NPY issues related to adipose-tissue

- Do alterations in NPY-mediated pathways play etiological roles in human anorexia and obesity?
- Can NPY-mediated pathways be targeted as treatment to correct body fat mass in human anorexia and obesity?
- Which Y receptor subtype(s) play major roles in mediating these effects in adipose tissues and can therapeutic treatment target the peripheral actions of NPY?

NPY and sex steroid-related issues

- Does increased action of the NPY system contribute to the bone loss and fat gain observed following the menopause?
- Do sex hormones and NPY interact in determining whether a mesenchymal stem cell will differentiate into a bone cell or an adipocyte? If yes, at what level does this regulation occur? Can we modify the interaction to control mesenchymal stem cell differentiation and in turn increase bone mass at the expense of fat mass?

Acknowledgments

This research was supported by grants from the National Health and Medical Research Council (NHMRC) of Australia and NHMRC Fellowships to H.H., P.A.B. and A.S. as well as an Australian Postgraduate Award (APA) Scholarship to A.Z.

References

1 Lee, N.K. *et al.* (2007) Endocrine regulation of energy metabolism by the skeleton. *Cell* 130, 456–469
2 Asarian, L. and Geary, N. (2006) Modulation of appetite by gonadal steroid hormones. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 361, 1251–1263
3 Clegg, D.J. *et al.* (2006) Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes* 55, 978–987
4 Riggs, B.L. *et al.* (2002) Sex steroids and the construction and conservation of the adult skeleton. *Endocr. Rev.* 23, 279–302
5 Davey, R.A. and Morris, H.A. (2004) Effects of dihydrotestrone on osteoblast gene expression in osteopenic ovariectomized rats. *Endocr. Res.* 30, 361–368
6 Notini, A.J. *et al.* (2007) Osteoblast deletion of exon 3 of the androgen receptor gene results in trabecular bone loss in adult male mice. *J. Bone Miner. Res.* 22, 347–356
7 Venken, K. *et al.* (2007) Impact of androgens, growth hormone, and IGF-I on bone and muscle in male mice during puberty. *J. Bone Miner. Res.* 22, 72–82
8 Vidal, O. *et al.* (1999) Expression and localization of estrogen receptor-beta in murine and human bone. *J. Bone Miner. Res.* 14, 923–929
9 Baldock, P.A. *et al.* (2002) Hypothalamic Y2 receptors regulate bone formation. *J. Clin. Invest.* 109, 915–921
10 Heilig, M. *et al.* (1989) Opposite effects of centrally administered neuropeptide Y (NPY) on locomotor activity of spontaneously hypertensive (SH) and normal rats. *Acta Physiol. Scand.* 137, 243–248
11 Kuo, L.E. *et al.* (2007) Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat. Med.* 13, 803–811
12 Teixeira, L. *et al.* (2009) NPY revealed as a critical modulator of osteoblast function in vitro: new insights into the role of Y1 and Y2 receptors. *J. Cell. Biochem.* 107, 908–916
13 Allison, S.J. *et al.* (2006) Conditional deletion of hypothalamic Y2 receptors reverts gonadectomy-induced bone loss in adult mice. *J. Biol. Chem.* 281, 23436–23444
14 Baldock, P.A. *et al.* (2007) Novel role of Y1 receptors in the coordinated regulation of bone and energy homeostasis. *J. Biol. Chem.* 282, 19092–19102
15 Gruber, C.J. *et al.* (2002) Production and actions of estrogens. *N. Engl. J. Med.* 346, 340–352
16 Vanderschueren, D. *et al.* (2004) Androgens and bone. *Endocr. Rev.* 25, 389–425
17 Rehman, H.U. and Masson, E.A. (2005) Neuroendocrinology of female aging. *Gend. Med.* 2, 41–56
18 Venken, K. *et al.* (2008) Sex hormones, their receptors and bone health. *Osteoporos. Int.* 19, 1517–1525
19 Malluche, H.H. *et al.* (1986) Osteoblastic insufficiency is responsible for maintenance of osteopenia after loss of ovarian function in experimental beagle dogs. *Endocrinology* 119, 2649–2654
20 Chow, J.W. *et al.* (1992) 17 beta-estradiol stimulates cancellous bone formation in female rats. *Endocrinology* 130, 3025–3032
21 Chow, J. *et al.* (1992) Estrogen maintains trabecular bone volume in rats not only by suppression of bone resorption but also by stimulation of bone formation. *J. Clin. Invest.* 89, 74–78
22 Eghbali-Fatourechi, G. *et al.* (2003) Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J. Clin. Invest.* 111, 1221–1230
23 Kawano, H. *et al.* (2003) Suppressive function of androgen receptor in bone resorption. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9416–9421
24 Kimble, R.B. *et al.* (1996) Estrogen deficiency increases the ability of stromal cells to support murine osteoclastogenesis via an interleukin-1and tumor necrosis factor-mediated stimulation of macrophage colony-stimulating factor production. *J. Biol. Chem.* 271, 28890–28897
25 Manolagas, S.C. and Jilka, R.L. (1995) Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N. Engl. J. Med.* 332, 305–311
26 Falahati-Nini, A. *et al.* (2000) Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. *J. Clin. Invest.* 106, 1553–1560
27 Leder, B.Z. *et al.* (2003) Differential effects of androgens and estrogens on bone turnover in normal men. *J. Clin. Endocrinol. Metab.* 88, 204–210
28 Chen, J.R. *et al.* (2005) Transient versus sustained phosphorylation and nuclear accumulation of ERKs underlie anti-versus pro-apoptotic effects of estrogens. *J. Biol. Chem.* 280, 4632–4638
29 Oursler, M.J. *et al.* (1994) Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. *Proc. Natl. Acad. Sci. U. S. A.* 91, 5227–5231
30 Kasperk, C.H. *et al.* (1997) Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro. *J. Bone Miner. Res.* 12, 464–471
31 Notelovitz, M. (2002) Androgen effects on bone and muscle. *Fertil. Steril.* 77 (Suppl 4), S34–S41
32 Vandenput, L. *et al.* (2001) Testosterone prevents orchidectomy-induced bone loss in estrogen receptor-alpha knockout mice. *Biochem. Biophys. Res. Commun.* 285, 70–76
33 Meier, C. *et al.* (2008) Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study. *Arch. Intern. Med.* 168, 47–54
34 Munoz-Torres, M. *et al.* (1995) Bone mass in androgen-insensitivity syndrome: response to hormonal replacement therapy. *Calcif. Tissue Int.* 57, 94–96
35 Ainslie, D.A. *et al.* (2001) Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. *Int. J. Obes. Relat. Metab. Disord.* 25, 1680–1688
36 Allison, S.J. *et al.* (2009) Critical interplay between neuropeptide Y and sex steroid pathways in bone and adipose tissue homeostasis. *J. Bone Miner. Res.* 24, 294–304
37 Lovejoy, J.C. and Sainsbury, A. (2009) Sex differences in obesity and the regulation of energy homeostasis. *Obes. Rev.* 10, 154–167
38 Poehlman, E.T. (2002) Menopause, energy expenditure, and body composition. *Acta Obstet. Gynecol. Scand.* 81, 603–611
39 Jacobsen, D.E. *et al.* (2007) Postmenopausal HRT and tibolone in relation to muscle strength and body composition. *Maturitas* 58, 7–18
40 Mattiasson, I. *et al.* (2002) Effects of estrogen replacement therapy on abdominal fat compartments as related to glucose and lipid metabolism in early postmenopausal women. *Horm. Metab. Res.* 34, 583–588
41 Cooke, P.S. and Naaz, A. (2004) Role of estrogens in adipocyte development and function. *Exp. Biol. Med. (Maywood)* 229, 1127–1135
42 D'Eon, T.M. *et al.* (2005) Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J. Biol. Chem.* 280, 35983–35991
43 Lovejoy, J.C. *et al.* (2008) Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int. J. Obes. (Lond)* 32, 949–958
44 Derby, C.A. *et al.* (2006) Body mass index, waist circumference and waist to hip ratio and change in sex steroid hormones: the Massachusetts Male Ageing Study. *Clin. Endocrinol. (Oxf)* 65, 125–131
45 Haffner, S.M. *et al.* (1993) Obesity, body fat distribution and sex hormones in men. *Int. J. Obes. Relat. Metab. Disord.* 17, 643–649
46 Blouin, K. *et al.* (2005) Contribution of age and declining androgen levels to features of the metabolic syndrome in men. *Metabolism* 54, 1034–1040
47 Lovejoy, J.C. *et al.* (1995) Oral anabolic steroid treatment, but not parenteral androgen treatment, decreases abdominal fat in obese, older men. *Int. J. Obes. Relat. Metab. Disord.* 19, 614–624
48 Marin, P. *et al.* (1993) Androgen treatment of abdominally obese men. *Obes. Res.* 1, 245–251
49 Mudali, S. and Dobs, A.S. (2004) Effects of testosterone on body composition of the aging male. *Mech. Ageing Dev.* 125, 297–304
50 Christoffersen, B. *et al.* (2006) Evalution of the castrated male Sprague-Dawley rat as a model of the metabolic syndrome and type 2 diabetes. *Int. J. Obes. (Lond)* 30, 1288–1297
51 Gupta, V. *et al.* (2008) Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. *Mol. Cell. Endocrinol.* 296, 32–40

52 Moverare-Skrtic, S. *et al.* (2006) Dihydrotestosterone treatment results in obesity and altered lipid metabolism in orchidectomized mice. *Obesity (Silver Spring)* 14, 662–672
53 Pasquali, R. (2006) Obesity and androgens: facts and perspectives. *Fertil. Steril.* 85, 1319–1340
54 Joyner, J. *et al.* (2002) Intrinsic regional differences in androgen receptors and dihydrotestosterone metabolism in human preadipocytes. *Horm. Metab. Res.* 34, 223–228
55 Blouin, K. *et al.* (2008) Androgens and body fat distribution. *J. Steroid Biochem. Mol. Biol.* 108, 272–280
56 Lemieux, S. *et al.* (1993) Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. *Am. J. Clin. Nutr.* 58, 463–467
57 Ducy, P. *et al.* (2000) Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 100, 197–207
58 Patel, M.S. and Elefteriou, F. (2007) The new field of neuroskeletal biology. *Calcif. Tissue Int.* 80, 337–347
59 Wong, I.P. *et al.* (2008) Central regulation of bone mass. *Semin. Cell Dev Biol* 19, 452–458
60 Baldock, P.A. *et al.* (2009) Neuropeptide Y knockout mice reveal a central role of NPY in the coordination of bone mass to body weight. *PLoS One* 4, e8415
61 Baldock, P.A. *et al.* (2005) Hypothalamic control of bone formation: distinct actions of leptin and y2 receptor pathways. *J. Bone Miner. Res.* 20, 1851–1857
62 Elefteriou, F. *et al.* (2003) Monosodium glutamate-sensitive hypothalamic neurons contribute to the control of bone mass. *Endocrinology* 144, 3842–3847
63 Reid, I.R. (2002) Relationships among body mass, its components, and bone. *Bone* 31, 547–555
64 Devogelaer, J.P. *et al.* (2006) Evidence-based guidelines for the prevention and treatment of glucocorticoid-induced osteoporosis: a consensus document of the Belgian Bone Club. *Osteoporos. Int.* 17, 8–19
65 Lin, E.J. *et al.* (2006) Combined deletion of Y1, Y2, and Y4 receptors prevents hypothalamic neuropeptide Y overexpression-induced hyperinsulinemia despite persistence of hyperphagia and obesity. *Endocrinology* 147, 5094–5101
66 Lin, S. *et al.* (2000) Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. *Brain Res.* 875, 89–95
67 Sainsbury, A. and Zhang, L. (2010) Role of the arcuate nucleus of the hypothalamus in regulation of body weight during energy deficit. *Mol. Cell Endocrinol.* 316, 109–116
68 Igwe, J.C. *et al.* (2009) Neuropeptide Y is expressed by osteocytes and can inhibit osteoblastic activity. *J. Cell Biochem.* 108, 621–630
69 Lundberg, P. *et al.* (2007) Greater bone formation of Y2 knockout mice is associated with increased osteoprogenitor numbers and altered Y1 receptor expression. *J. Biol. Chem.* 282, 19082–19091
70 Serradeil-Le Gal, C. *et al.* (2000) Characterization of NPY receptors controlling lipolysis and leptin secretion in human adipocytes. *FEBS Lett.* 475, 150–156
71 Yang, K. *et al.* (2008) Neuropeptide Y is produced in visceral adipose tissue and promotes proliferation of adipocyte precursor cells via the Y1 receptor. *FASEB J.* 22, 2452–2464
72 Labelle, M. *et al.* (1997) Tissue-specific regulation of fat cell lipolysis by NPY in 6-OHDA-treated rats. *Peptides* 18, 801–808
73 Sainsbury, A. *et al.* (2003) Synergistic effects of Y2 and Y4 receptors on adiposity and bone mass revealed in double knockout mice. *Mol. Cell. Biol.* 23, 5225–5233
74 Wortley, K.E. *et al.* (2007) Peptide YY regulates bone turnover in rodents. *Gastroenterology* 133, 1534–1543
75 Misra, M. *et al.* (2006) Elevated peptide YY levels in adolescent girls with anorexia nervosa. *J. Clin. Endocrinol. Metab.* 91, 1027–1033
76 Cerda-Reverter, J.M. and Larhammar, D. (2000) Neuropeptide Y family of peptides: structure, anatomical expression, function, and molecular evolution. *Biochem. Cell Biol.* 78, 371–392
77 Bard, J.A. *et al.* (1995) Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. *J. Biol. Chem.* 270, 26762–26765
78 File, E. and Deal, C. (2009) Clinical update on teriparatide. *Curr. Rheumatol. Rep.* 11, 169–176
79 Andersson, N. *et al.* (2005) Investigation of central versus peripheral effects of estradiol in ovariectomized mice. *J. Endocrinol.* 187, 303–309
80 Clegg, D.J. *et al.* (2007) Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats. *Diabetes* 56, 1051–1058
81 Hofbauer, L.C. *et al.* (1999) Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrinology* 140, 4367–4370
82 Blomqvist, A.G. and Herzog, H. (1997) Y-receptor subtypes – how many more? *Trends Neurosci.* 20, 294–298
83 Michel, M.C. *et al.* (1998) XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol. Rev* 50, 143–150
84 Chronwall, B.M. *et al.* (1985) The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15, 1159–1181
85 de Quidt, M.E. and Emson, P.C. (1986) Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system. II. Immunohistochemical analysis. *Neuroscience* 18, 545–618
86 Ekblad, E. *et al.* (1984) Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul. Pept.* 8, 225–235
87 Lundberg, J.M. *et al.* (1982) Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiol. Scand.* 116, 477–480
88 Upchurch, B.H. *et al.* (1996) Peptide YY expression is an early event in colonic endocrine cell differentiation: evidence from normal and transgenic mice. *Development* 122, 1157–1163
89 Larsson, L.I. *et al.* (1976) Pancreatic polypeptide – a postulated new hormone: identification of its cellular storage site by light and electron microscopic immunocytochemistry. *Diabetologia* 12, 211–226
90 Henry, M. *et al.* (2005) Energy metabolic profile of mice after chronic activation of central NPY Y1, Y2, or Y5 receptors. *Obes. Res.* 13, 36–47
91 Asakawa, A. *et al.* (2003) Characterization of the effects of pancreatic polypeptide in the regulation of energy balance. *Gastroenterology* 124, 1325–1336
92 Balasubramaniam, A. *et al.* (2006) Neuropeptide Y (NPY) Y4 receptor selective agonists based on NPY(32-36): development of an anorectic Y4 receptor selective agonist with picomolar affinity. *J. Med. Chem.* 49, 2661–2665
93 Batterham, R.L. *et al.* (2003) Inhibition of food intake in obese subjects by peptide YY3-36. *N. Engl. J. Med.* 349, 941–948
94 Batterham, R.L. *et al.* (2002) Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 418, 650–654
95 Batterham, R.L. *et al.* (2003) Pancreatic polypeptide reduces appetite and food intake in humans. *J. Clin. Endocrinol. Metab.* 88, 3989–3992
96 Jesudason, D.R. *et al.* (2007) Low-dose pancreatic polypeptide inhibits food intake in man. *Br. J. Nutr.* 97, 426–429
97 Pittner, R.A. *et al.* (2004) Effects of PYY3-36 in rodent models of diabetes and obesity. *Int J. Obes. Relat. Metab. Disord.* 28, 963–971
98 Zhang, L. *et al.* (2010) Peripheral neuropeptide Y Y1 receptors regulate lipid oxidation and fat accretion. *Int. J. Obes. (Lond)* 34, 357–373
99 Bischoff, A. and Michel, M.C. (1998) Renal effects of neuropeptide Y. *Pflugers Arch.* 435, 443–453
100 Wheway, J. *et al.* (2007) NPY and receptors in immune and inflammatory diseases. *Curr. Top. Med. Chem.* 7, 1743–1752
101 Edelsbrunner, M.E. *et al.* (2009) Evidence from knockout mice that peptide YY and neuropeptide Y enforce murine locomotion, exploration and ingestive behaviour in a circadian cycle- and gender-dependent manner. *Behav. Brain Res.* 203, 97–107
102 Yannielli, P.C. and Harrington, M.E. (2001) Neuropeptide Y in the mammalian circadian system: effects on light-induced circadian responses. *Peptides* 22, 547–556
103 Catzeflis, C. *et al.* (1993) Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotropic axis in intact adult female rats. *Endocrinology* 132, 224–234
104 Pierroz, D.D. *et al.* (1996) Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. *Endocrinology* 137, 3–12
105 Gonzales, C. *et al.* (2004) The neuropeptide Y Y1 receptor mediates NPY-induced inhibition of the gonadotrope axis under poor metabolic conditions. *FASEB J.* 18, 137–139
106 Sainsbury, A. *et al.* (2002) Y4 receptor knockout rescues fertility in *ob/ob* mice. *Genes Dev.* 16, 1077–1088