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Role of the hypothalamus in the neuroendocrine regulation of body weight and composition during energy deficit

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Summary

Energy deficit in lean or obese animals or humans stimulates appetite, reduces energy expenditure and possibly also decreases physical activity, thereby contributing to weight regain. Often overlooked in weight loss trials for obesity, however, is the effect of energy restriction on neuroendocrine status. Negative energy balance in lean animals and humans consistently inhibits activity of the hypothalamo-pituitary-thyroid, -gonadotropic and -somatotropic axes (or reduces circulating insulin-like growth factor-1 levels), while concomitantly activating the hypothalamo-pituitary-adrenal axis, with emerging evidence of similar changes in overweight and obese people during lifestyle interventions for weight loss. These neuroendocrine changes, which animal studies show may result in part from hypothalamic actions of orexigenic (e.g. neuropeptide Y, agouti-related peptide) and anorexigenic peptides (e.g. alpha-melanocyte-stimulating hormone, and cocaine and amphetamine-related transcript), can adversely affect body composition by promoting the accumulation of adipose tissue (particularly central adiposity) and stimulating the loss of lean body mass and bone. As such, current efforts to maximize loss of excess body fat in obese people may inadvertently be promoting long-term complications such as central obesity and associated health risks, as well as sarcopenia and osteoporosis. Future weight loss trials would benefit from assessment of the effects on body composition and key hormonal regulators of body composition using sensitive techniques.

Introduction

A major challenge in the treatment of obesity is that the human body responds to energy restriction and weight loss with a diverse range of adaptive responses that oppose ongoing weight loss and promote regain. Such changes include robust increases in appetite, marked reductions in energy expenditure, and new studies also suggest reductions in physical activity **(1,2)** and the energy cost of activity **(3,4)**, as recently reviewed **(5–7)**. It is commonly assumed among healthcare professionals, the weight loss industry and members of the public alike that such energy-conserving adaptations only occur after extensive or rapid weight loss in lean individuals, as in the Minnesota starvation study published in 1950 **(8)**. However, such adaptations have been shown to occur even in overweight and obese people after loss of as little as 6–12% of body weight **(1,6,9–12)**, and even when weight loss is achieved using moderate energy restriction, with or without physical activity **(1,9,10)**. As the extent of the weight-loss-induced increases in appetite **(13,14)** or reductions in energy expenditure **(13–16)** predict subsequent weight regain, and as low levels of non-exercise activity thermogenesis also predict subsequent weight gain in rodents, monkeys and possibly also in humans **(7)**, it is likely that these adaptive responses contribute to the low success rate of non-surgical treatments for overweight and obesity.

Besides effects on energy intake, physical activity and energy expenditure, energy deficit has marked effects on the levels of hormones that affect body composition. Studies in lean animals and humans clearly show that negative energy balance markedly inhibits activity of the hypothalamo-pituitary-thyroid, -gonadotropic and -somatotropic axes (or reduces insulin-like growth factor-1 [IGF-1] levels), while concomitantly activating the hypothalamo-pituitary-adrenal axis. There is little information available as to the effects of weight loss in overweight or obese people on the circulating concentrations of effector hormones of these neuroendocrine axes (notably thyroid hormones, sex hormones, IGF-1 and cortisol), but available evidence suggests that similar changes to those occurring during energy deficit in lean animals and humans also occur in overweight and obese people during weight loss interventions. Such findings have important implications for the clinical management of obesity, as well as for the experimental use of calorie restriction to delay the onset of disease or death in humans **(5)**. For

instance, while dietary restriction in people who are overweight or obese certainly reduces body weight and adiposity in the initial months of application, it may also induce a neuroendocrine status that negatively affects body composition (e.g. by promoting increased central adiposity, reducing muscle mass and compromising bone mineral density), thereby inadvertently increasing the risk of metabolic diseases such as diabetes and atherosclerosis, as well as that of structural diseases such as sarcopenia and osteoporosis. Knowing the extent of any such neuroendocrine changes would enable weight management or calorie restriction methods to be optimized for maximum fat loss and health gains with minimum loss of tissues such as muscle and bone which are so important for healthy ageing.

This narrative review examines the effects of energy restriction on the hypothalamo-pituitary-thyroid, -adrenal, -gonadotropic and -somatotropic axes, spanning studies in lean rodents and humans to weight loss interventions in overweight and obese adults. Because understanding the mechanisms of such changes can inform the development of better weight loss interventions, the review then focuses on the possible role of the hypothalamus in mediating these processes during energy deficit, focusing predominantly on four major neurotransmitters whose expression is known to be altered in negative energy balance: neuropeptide Y (NPY), agouti-related peptide (AgRP), alpha-melanocyte-stimulating hormone (α-MSH), and cocaine and amphetamine-related transcript (CART). We focus on these particular neurotransmitters on account of our research experiences, and it must be pointed out that there are dozens of neuropeptides whose hypothalamic expression is altered by changes in energy balance and whose central administration results in short- or long-term changes in body weight or the parameters that influence it. Such factors include orexigenic signals such as galanin, melanin-concentrating hormone, glutamate, γ-aminobutyric acid, hypocretins/orexins, and the anorexigenic oxytocin and the corticotropin-releasing hormone (CRH) family of peptides as well as neurotensin **(17,18)**. Discussion of the possible role of these neuropeptides in the regulation of neuroendocrine status with energy deficit will not be covered in this review.

NPY is an orexigenic peptide **(19,20)** synthesized by neurons within the arcuate nucleus of the hypothalamus **(21)**. NPY and the other members of the NPY family – the gut-derived satiety hormones peptide YY and pancreatic polypeptide – exert their effects via at least five known Y receptors (Y1, Y2, Y4, Y5 and Y6) **(22)**. Y receptors are differentially expressed throughout the body, notably in the nervous system and gut, and NPY, peptide YY and pancreatic polypeptide have differing Y receptor-binding affinities **(22)**. Besides NPY, the central NPY-ergic neurons synthesize another orexigenic agent, AgRP **(23)**, which antagonizes melanocortin 3 (MC3) and melanocortin 4 (MC4) receptors expressed in the brain **(23)**. MC3 and MC4 receptors exhibit differential expression patterns throughout peripheral tissues or the brain, and they further differ from each other in their affinities for endogenous ligands **(24)**. Despite these differences, both MC3 and MC4 receptors are implicated in regulating various aspects of energy homeostasis **(24)**. The NPY-AgRP synthesizing neurons in the arcuate nucleus are distinct from the cells that express pro-opiomelanocortin (POMC) **(25)**, the precursor for the anorexigenic α-MSH, which acts as an agonist on brain MC3 and MC4 receptors **(26,27)**. NPY-AgRP and POMC-expressing neurons in the arcuate nucleus project to the paraventricular nucleus (PVN) **(28,29)**, where they exert stimulatory (NPY and AgRP) **(19,20)** or inhibitory (α-MSH) **(30,31)** effects on food intake. CART has been shown to have anorexigenic as well as orexigenic effects via actions in the hypothalamus **(32)**. During energy restriction in rodents, the protein or mRNA levels of NPY and AgRP are increased in the hypothalamus whereas the mRNA expression of POMC and CART are reduced, as recently reviewed **(6)**. This review focuses on how such hypothalamic neuropeptide changes could contribute to the observed alterations in neuroendocrine status seen during negative energy balance. Our review then synthesizes the effects that such changes in active levels of thyroid hormones, glucocorticoids, sex hormones and IGF-1 would have on body composition, drawing on studies in rodents and humans and with a focus on effects on total and central adiposity, muscle mass and bone mineral content or density. Lastly, we call for consideration of effects of various methods of weight loss on neuroendocrine status and body composition, so that treatments for obesity can be optimized not only for weight loss and fat loss, but also for optimum preservation of body composition during the weight loss and weight maintenance phases.

Effects of energy deficit and the resultant hypothalamic changes on neuroendocrine axes

In addition to stimulation of appetite, possible effects on physical activity and the energy cost of activity, as well as reduction in metabolic rate or energy expenditure **(1–7)**, energy deficit results in neuroendocrine alterations that could collectively inhibit further fat loss and promote loss of lean tissues such as muscle and bone, as described below and as summarized in Fig. 1.

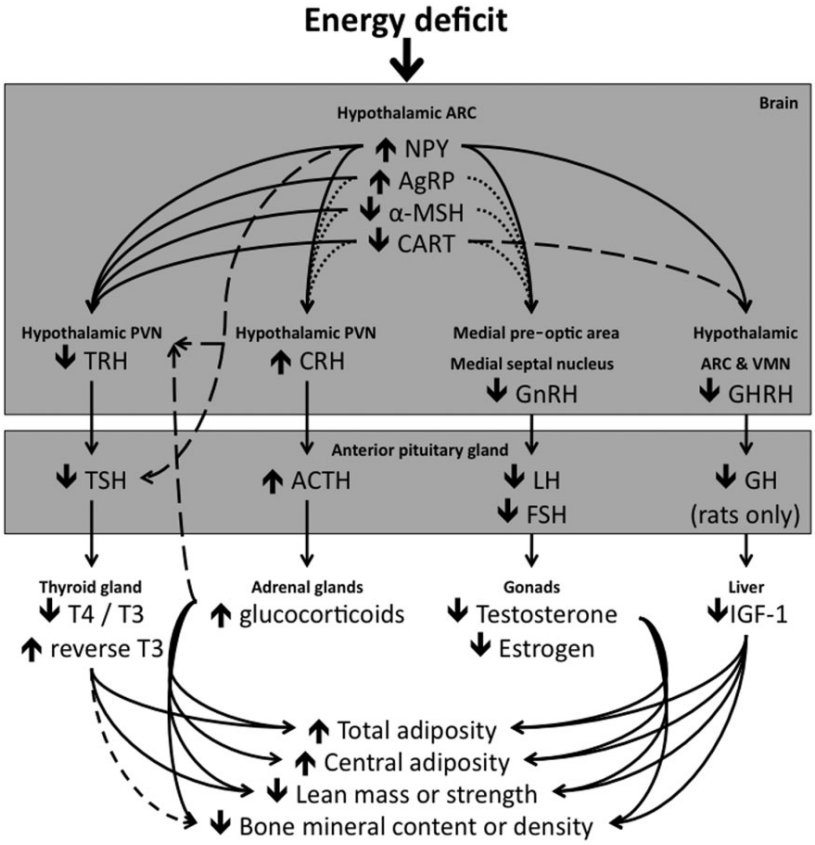

Figure 1 Energy deficit in lean and possibly also obese animals or humans induces effects on hypothalamo-pituitary axes that would be expected to promote total and central adiposity, decrease lean mass or muscle strength and reduce bone mineral content or density. These neuroendocrine effects of energy deficit may be mediated via changes in the hypothalamic arcuate nucleus (ARC): increased expression of orexigenic peptides such as neuropeptide Y (NPY) and agouti-related peptide (AgRP), and reduced expression of anorexigenic peptides such as alpha-melanocyte-stimulating hormone (α-MSH) and cocaine and amphetamine-related transcript (CART), which has both orexigenic and anorexigenic properties. In addition to promoting food intake, inhibiting physical activity (or the energy cost of physical activity) and reducing energy expenditure (6), adaptations that have all been observed in lean and obese animals and humans in response to energy deficit (6), these changes in orexigenic and anorexigenic peptide expression have multiple effects on the hypothalamus. They collectively inhibit expression or release of thyrotropin-releasing hormone (TRH) in the hypothalamic paraventricular nucleus (PVN), as well as reducing that of gonadotropin-releasing hormone (GnRH) and – at least in rats – growth hormone-releasing hormone (GHRH) in the ARC or hypothalamic ventromedial nucleus (VMN), while stimulating that of corticotropin-releasing hormone (CRH) in the PVN. These changes in turn inhibit anterior pituitary secretion of thyroid-stimulating hormone (TSH), the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and – in rats but not mice or humans – growth hormone (GH), with a concomitant increase in anterior pituitary adrenocorticotropic hormone (ACTH) output. The result of these changes is inhibition of thyroid function (e.g. by reducing the secretion of triiodothyronine or 3,3′,5-triiodothyronine [T3] and its less active precursor thyroxine or 3,5,3′,5′-tetraiodothyronine [T4] from the thyroid gland, reducing the tissue conversion of T4 to T3, and enhancing the tissue conversion of T4 to reverse T3, which blocks the action of thyroid hormones at thyroid hormone receptors), enhanced output of glucocorticoids from the adrenal glands, reduced production or biological activity of gonadal sex steroids, notably testosterone and oestradiol, and – in mice, rats and humans – reduction in the hepatic production of insulin-like growth factor-1 (IGF-1). As there is some evidence that these hormonal changes may occur in overweight or obese people in response to energy restriction, and as these hormonal changes have been shown to alter metabolic processes to favour fat accretion (particularly central fat accretion), and to enhance loss of lean tissue or bone, it is important to consider the long-term consequences of emerging weight loss strategies on regional body composition using sensitive techniques such as magnetic resonance imaging or computerized tomography. Please see text for references. Arrows denote strongly supported effects, not necessarily via direct neuronal connections. Finely dotted arrows (e.g. from AgRP to CRH) represent possible transient stimulatory or inhibitory effects. Dashed arrows (e.g. from NPY to TSH) represent possible effects.

Inhibition of the hypothalamo-pituitary-thyroid axis

Thyroid hormones (T3, otherwise known as triiodothyronine or 3,3′,5-triiodothyronine, and its less active precursor T4, otherwise known as thyroxine or 3,5,3′,5′-tetraiodothyronine) are major regulators of energy expenditure and a key pathway for hypothalamic regulation of energy balance. During energy deficit, thyroid hormone function is inhibited at multiple levels of the hypothalamo-pituitary-thyroid axis.

Changes in thyroid function with energy restriction in animals

The function of the hypothalamo-pituitary-thyroid axis is controlled by thyrotropin-releasing hormone (TRH). TRH is synthesized in the PVN, released into the hypophyseal portal system and transported to the pars distalis in the anterior lobe of the pituitary gland where it stimulates the release of thyrotropin, otherwise known as thyroid-stimulating hormone (TSH). TSH in turn stimulates the synthesis and release of thyroid hormones from the thyroid gland. Fasting in rats induces a significant reduction in the expression of TRH in the PVN **(33)**, and this probably contributes to the significant reductions in circulating T3 and/or T4 levels seen in male rats and mice of both genders after a 24- to 48-h fast **(34–36)**.

Thyroid hormone conversion is another important point through which energy deficit regulates thyroid function. The major thyroid hormone produced by the thyroid gland and found in the circulation is T4, and this is converted within cells to the three- to fourfold more active hormone T3 by the action of 5′-deiodinases as recently reviewed **(37)**. In contrast, 5-deiodinases result in formation of 3,3,5′-triiodothyronine (reverse T3) which does not stimulate thyroid hormone receptors but instead binds to these receptors and blocks their activation by thyroid hormones. Most vertebrate species express three deiodinase enzymes, deiodinases 1, 2 and 3 (DIO1-3). DIO1 can function as both a 5′-deiodinase and a 5-deiodinase and is most highly expressed in the adult liver, kidney and thyroid gland. DIO2, which is exclusively a 5'-deiodinase, is expressed principally in the brain, brown adipose tissue, pituitary gland, and other endocrine and reproductive organs. DIO3 functions as a pure 5-deiodinase and, in the adult, is most highly expressed in the brain, skin, and pregnant uterus and placenta **(38)**. The activity and/or mRNA levels of hepatic DIO1 and pituitary DIO2 have been shown to be decreased in male rats or female mice after a 24-h fast **(35,39)**, and this could contribute to the reduced conversion of T4 to the more active hormone T3 that has been observed in liver homogenates from 72-h fasted male rats **(40)**. Not only does fasting decrease conversion of T4 to T3 in rat liver homogenates, it also reduces conversion of T4 to the inactive hormone reverse T3 **(40)**. However, the *ratio* of conversion of T4 to T3 vs. reverse T3 is reduced by fasting **(40)**, thereby contributing to reduced thyroid function.

The hypothalamo-pituitary-thyroid axis undergoes negative feedback regulation, with thyroid hormones inhibiting the axis at the level of TRH in the hypothalamus and TSH in the anterior pituitary. Therefore, the low circulating thyroid hormone levels seen in fasting might be expected to enhance TRH expression, but this is not the case. This apparent paradox is likely because while fasting decreases T4 conversion in peripheral tissues such as liver in association with decreased expression and activity of DIO1, it *increases* DIO2 expression in the hypothalamus leading to elevation of hypothalamic T3 levels during fasting **(41,42)**. This localized increase in T3 likely contributes to sustained down-regulation of hypothalamic TRH expression during fasting, because when this change is blocked by inhibition of DIO2, the fasting-induced decrease in hypothalamic TRH expression is no longer apparent **(42)**. The fasting-induced increase in hypothalamic T3 levels likely contributes to the increased appetite associated with fasting, via T3-triggered activation of uncoupling protein 2 in neuronal mitochondria, in turn enabling increased excitability of orexigenic NPY/AgRP neurons in the arcuate nucleus **(43)**.

In addition to regulation by production of active thyroid hormones, energy deficit down-regulates the binding of thyroid hormones to their receptors. There are at least four isoforms of thyroid hormone receptors (α1, α2, β1 and β2) which – typical of steroid hormone receptors – travel to the nucleus and bind to specific DNA elements to regulate gene transcription. In male rats, 48-h fasting results in marked and significant reductions in the maximal binding capacity of thyroid hormone receptors in liver and in different tissues **(36)**. Additionally, 24-h fasting has been shown to significantly decrease mRNA levels of thyroid hormone receptor β2 mRNA in the pituitary gland of female mice **(35)**. These changes can further contribute to reduced thyroid

function under conditions of inadequate energy intake in rodents.

Changes in thyroid function with energy restriction in humans

Similar to the changes in thyroid function seen in lean rodents after short-term fasting, lean healthy men and women also show profound fasting-induced inhibition of the hypothalamo-pituitary-thyroid axis by several mechanisms operating at various organ levels as recently reviewed **(44)**. Such changes include decreased TSH release from the anterior pituitary, decreased secretion of thyroid hormones from the thyroid gland and alterations in conversion of T4. The result of these changes are reduced circulating concentrations of T3, increased circulating concentrations of the inactive hormone reverse T3, with either reduction or no change in the circulating concentrations of TSH, T4 and free T4 **(45)**. Consistent with the effects of energy deficiency on thyroid function, people with anorexia nervosa typically exhibit low circulating levels of T4 and/or T3 and elevated levels of reverse T3, with normal or slightly reduced circulating TSH levels, suggesting central mediation **(45)**. In keeping with reduced thyroid function, anorexia nervosa is associated with many of the clinical features of hypothyroidism, including hypothermia and bradycardia **(45)**. There is also some evidence that thyroid function is inhibited in elite athletes, as indicated by reduced circulating concentrations of T3 or increased circulating reverse T3 levels **(46,47)**. Such a change is consistent with the relative energy deficit commonly observed in elite athletes, especially among those participating in activities such as gymnastics where a low body fat mass is highly prized **(47)**.

While thyroid function is inhibited by severe short-term caloric restriction in lean humans, weight loss by less severe energy restriction in overweight or obese individuals also leads to significant reductions in functions of the hypothalamo-pituitary-thyroid axis, and this probably contributes to the significant reductions in metabolic rate and energy expenditure that have been observed in these people **(6,48)**. Overweight or obese men and women who lost weight by a very low energy diet or even by moderate energy restriction (with or without physical activity) generally showed significant decreases in circulating concentrations of the most active thyroid hormone free T3 and/or a significant increase in that of the inactive hormone, reverse T3, as well as no change or significant reductions in circulating concentrations of TSH **(12,48–54)**. The serum concentrations of T4 or free T4, the less active precursor to T3, were either decreased, unchanged or increased by energy restriction in overweight or obese people **(12,48–54)**. As shown in Table 1, a summary of original papers from which these findings are drawn, the overall pattern observed is a decrease in thyroid function with energy restriction in overweight or obese people.

Effects of hypothalamic regulators of energy balance on the thyroid axis

It is likely that the changes in activity of the hypothalamo-pituitary-thyroid axis seen with energy deficit as described above are at least partially mediated by the hypothalamic changes induced by energy deficiency, as summarized in Fig. 1. For instance, enhanced hypothalamic expression of the orexigenic peptides NPY and AgRP with energy deficiency **(6)** likely inhibits the thyroid axis, because central administration of NPY, AgRP or MC4 receptor antagonists to male rats significantly reduces function of the thyrotropic axis, indicated by reductions in expression of TRH in the PVN, and reductions in circulating concentrations of TSH and T3 or T4 **(55–57)**. Neuronal endings containing NPY- or AgRP-immunoreactivity have been detected in close association with the TRH-synthesizing neurons of the hypothalamic PVN in rodents **(58,59)** and in men and women **(60)**, suggesting direct inhibitory effects of NPY and AgRP on the thyrotropic axis at the level of the hypothalamus. Additionally, NPY may inhibit the hypothalamo-pituitary-thyroid axis by direct action on the pituitary gland, because NPY dose dependently inhibited mRNA levels of the TSHβ subunit in pituitary cells *in vitro***(61)**.

In addition to up-regulation of hypothalamic NPY and AgRP expression, energy deficiency increases activity of the hypothalamo-pituitary-adrenal axis as described below (see Activation of the hypothalamo-pituitary-adrenal axis), and this change may also contribute to inhibition of the thyroid axis under conditions of inadequate energy intake. Adrenalectomy in rats increases pro-TRH mRNA levels in the PVN, whereas administration of exogenous corticosterone or dexamethasone to rats has the opposite effect, resulting in inhibition of pro-TRH mRNA in the PVN **(62)**. Similar effects appear to be at play in humans, since people that had been treated with corticosteroids until the time of death showed a significant decrease in TRH mRNA levels in the PVN compared to control subjects **(63)**, and glucocorticoid excess has been shown to suppress the secretion of TSH in humans **(64)**. These effects may occur via direct action

Table 1 Summary of original publications showing effects of energy restriction on function of the hypothalamo-pituitary-thyrotropic, -corticotropic, -gonadotropic and -somatotropic axes in overweight or obese people

Publication details	Energy restriction	Change from baseline	Notes
		Thyrotropic axis	
12 overweight men (12)	8–46 d on VLED	⇔ TSH	Measured only after 25 d. Tendency to be decreased.
		⇑ Free T4	
		⇓ Free T3	
18 overweight and obese women (49)	20% for 28 d then 50% for additional 28 d	⇓ TSH	
		⇓ T4	
		⇔ Free T4	
		⇓ T3	Greater decrease after 56 d
		⇓ T3/T4 ratio	Only after 56 d
6–10 obese men and women (20% men) (50)	42–70 d on VLED until 10% or 20% weight loss	⇔ TSH	
		⇔ T4	
		⇓ T3	Only after 20% weight reduction
		⇑ Reverse T3	Only after 20% weight reduction
24 overweight women (51)	VLED until loss of ≥10 kg and reaching BMI < 25 kg m^{-2}	⇓ T3	In early phase of energy restriction
		⇑ Reverse T3	
		⇓ T3/reverse T3 ratio	
15 obese women (52)	56 d on VLED ($n = 8$) vs. 1,200 kcal d^{-1} diet ($n = 7$) (after lead-in on 28 d on 1,200 kcal diet)	⇓ T3	Up to 66% reduction after VLED, up to 40% reduction after less restrictive diet
		⇑ Reverse T3	Only after VLED
44 obese men (53)	1 and 2 years on group lifestyle programme aiming for 1,600 kcal d^{-1} and regular physical activity	⇓ TSH	
		⇑ Free T4	
		⇔ Free T3	
		⇔ Free T3/Free T4 ratio	
		Corticotropic axis	
6 obese men (84)	6 d fasting plus 4 structured exercise sessions	⇑ Cortisol	Morning and evening
		⇔ Urinary cortisol metabolite excretion	Features consistent with central activation of the corticotropic axis
		⇑ Urinary free cortisol	
		⇑ 5β- rather than 5α-reduction of cortisol	
6 obese men (84)	21 d on VLED plus 40 min cycling per day	⇔ Cortisol	
		⇓ Cortisol production and metabolism of cortisol and cortisone	
12 overweight men (12)	8–46 d on VLED	⇔ Cortisol	Tendency to be increased
25 obese people (85)	84 d at 30% energy restriction	⇔ 24-h urinary free cortisol/cortisone	
		⇔ Basal or ACTH-stimulated free and total cortisol or CBG	
10 obese men, 7 obese women (9)	105 d at restriction of –2,930 kJ d^{-1} (–700 kcal d^{-1})	Increased appetite consistently predicted by fasting plasma cortisol levels	
		Gonadotropic axis	
10 overweight men (12)	25 d on VLED	⇓ Prolactin	
		⇓ LH	
		⇔ FSH	Tendency to be decreased
		⇔ Total testosterone	
		⇑ SHBG	
		⇓ Free testosterone	
6 overweight and obese men (128)	10 d fast	⇔ LH	
		⇓ FSH	
		⇓ FSH response to GnRH	
		⇑ 24-h urinary excretion of LH and FSH	
		⇓ Testosterone	
58 obese men (129)	63 d on VLED	⇑ Total testosterone	
		⇑ SHBG	
		⇑ Free testosterone	
		Somatotropic axis	
12 overweight men (12)	8–46 d on VLED	⇑ GH	Measured only after 25 d
		⇓ IGF-1	

All changes refer to circulating concentrations unless otherwise stated.
ACTH, adrenocorticotropic hormone; BMI, body mass index; CBG, corticosteroid-binding globulin; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; IGF-1, insulin-like growth factor-1; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; T3, triiodothyronine or 3,3′,5-triiodothyronine; T4, thyroxine or 3,5,3′,5′-tetraiodothyronine; TSH, thyroid-stimulating hormone; VLED, very low energy diet.

on TRH-expressing neurons in the PVN, because glucocorticoid receptor-binding site consensus sequences have been identified in the TRH gene **(65)**. In addition to regulation by glucocorticoids, the thyroid axis may also be regulated by CRH, the major regulator of the hypothalamo-pituitary-adrenal axis, because CRH-immunoreactive neurons make reciprocal synaptic connections with TRH-immunoreactive neurons in the PVN of rats **(66,67)**, and

application of CRH to rat hypothalami *in vitro* leads to inhibition of immunoreactive TRH release **(68)**, albeit another study has shown an increase in TRH mRNA levels in cultures of rat hypothalamic cells after incubation with CRH **(69)**. Taken together, these findings show that activity of the hypothalamo-pituitary-adrenal axis can down-regulate that of the hypothalamo-pituitary-thyroid axis, and this may occur via direct actions of glucocorticoids and possibly also CRH on hypothalamic TRH-synthesizing neurons. These effects may contribute to the propensity for weight gain that have been observed both after negative energy balance when the adrenal axis is stimulated (see Activation of the hypothalamo-pituitary-adrenal axis), and in cases of exogenous glucocorticoid treatment or Cushing's syndrome (see Effects of activation of the hypothalamo-pituitary-adrenal axis on body weight and composition).

In contrast to the inhibitory effects that NPY, AgRP and glucocorticoids have on the hypothalamo-pituitary-thyroid axis, the anorexigenic substances α-MSH and CART stimulate this axis. It has been shown that α-MSH or an α-MSH analogue increased plasma TSH and free T4 concentrations after intracerebroventricular (ICV) administration to rats, prevented the fasting-induced reduction in pro-TRH expression in the PVN and increased TRH release from hypothalamic explants *in vitro***(57,70)**. This regulation is likely to be directly mediated by α-MSH in the hypothalamus, which is contained in nerve terminals innervating TRH-synthesizing neurons in the PVN in rats **(70)** and in men and women **(60)**. Nerve terminals with CART immunoreactivity show numerous synaptic contacts with almost all pro-TRH mRNA-containing neurons in the rat PVN, and ICV administration of CART to rats completely prevented the fasting-induced inhibition of pro-TRH mRNA in the PVN **(71)**. *In vitro* data show similar effects, with CART increasing the content of TRH in hypothalamic cell cultures **(71)** and stimulating TRH secretion from hypothalamic explants *in vitro***(72)**. In addition to being innervated by CART-containing neurons, TRH-expressing neurons in the PVN co-express CART, albeit the functional significance of this for regulation of the hypothalamo-pituitary-thyroid axis is unclear given that CART has no effect on basal or TRH-stimulated release of TSH from anterior pituitary cells *in vitro* as recently reviewed **(73)**.

Activation of the hypothalamo-pituitary-adrenal axis

In addition to reducing activity of the thyroid axis, energy restriction activates the hypothalamo-pituitary-adrenal axis, which is predominantly controlled by CRH in the PVN. Stressful conditions increase the expression of CRH, which acts on CRH receptors 1 and 2 (CRH-R1 and CRH-R2) **(74)**. Activation of CRH-R1 has been connected to anxiogenic responses, while activation of CRH-R2 to anxiolytic responses **(74)**. Administration of exogenous CRH to rodents induces stress-like behaviours **(75)**. In addition, CRH mediates protection against stressful stimuli by activating the adrenal axis **(74)**. Like TRH, CRH is released into the hypophyseal portal system and is transported to the pars distalis in the anterior lobe of the pituitary gland, where it stimulates the release of adrenocorticotropic hormone (ACTH). ACTH in turn acts on the cortex of the adrenal glands to stimulate the production of corticosteroid hormones and sex hormones. Energy restriction has been shown to affect this axis at several levels as detailed below and as summarized in Fig. 1.

Changes in adrenal axis function with energy restriction in animals

Short-term (48-h) fasting in male mice has been clearly shown to increase circulating corticosterone concentrations **(34,76)**. This change may be mediated within the hypothalamus, because short-term fasting in male rats increases CRH mRNA levels in the PVN **(77)**. In addition to short-term fasting, longer-term energy restriction in rodents increases activity of the hypothalamo-pituitary-adrenal axis. When rats are given access to food for just 1–2 h d–1, and are given access to an activity wheel for the remainder of the day, energy expenditure exceeds energy intake and – if the experiment is not stopped by the investigator – the animals starve to death **(78)**. This paradigm has been used as a model of anorexia nervosa. When these meal fed-wheel running rats were studied after having lost 25% of their pre-experimental body weight, both male and female rats showed significant increases in circulating corticosterone concentrations and adrenal gland weight in the absence of any change in CRH mRNA levels in the PVN **(79)**.

Changes in adrenal axis function with energy restriction in humans

Unanimous research shows that energy restriction in normal weight or underweight humans

leads to up-regulation of the hypothalamo-pituitary-adrenal axis. Anorexia nervosa is associated with CRH-driven hyperactivity of the hypothalamo-pituitary-adrenal axis, as indicated by an increased 24-h mean plasma cortisol, an increased 24-h excretion of urinary free cortisol and failure of dexamethasone to suppress plasma ACTH and cortisol levels as previously reviewed **(45,80)**. These changes appear to be due to energy restriction/weight loss *per se* and not the underlying pathology causing anorexia nervosa, because similar stimulation of the hypothalamo-pituitary-adrenal axis (i.e. reduced suppression of circulating cortisol levels by dexamethasone, increased 24-h circulating cortisol levels and increased cortisol half-life) can be recapitulated in normal women by 3 weeks of complete abstinence from food **(81)**. It has been observed that highly trained athletes demonstrate chronic hypercortisolism **(46)**. Additionally, salivary cortisol levels were found to be elevated – and circadian rhythmicity in this parameter abolished – in young male and female artistic gymnasts relative to age-matched non-athletes **(82)**. While increased psychological stress likely contributed to the observed increase in salivary cortisol in these gymnasts **(82)**, energy deficit was another likely contributor. In keeping with this, in lean young men, an 8-week programme consisting of high volumes of physical activity and food restriction also resulted in marked weight loss in association with significant increases in serum cortisol **(83)**.

In addition to effects in lean and underweight people, a limited number of studies – listed in Table 1– have suggested that negative energy balance in obese people may increase circulating cortisol levels. Fasting for 6 d in obese men significantly increased morning and evening plasma cortisol levels **(84)**. Another study in overweight men showed that 46 d on a very low energy diet tended to increase circulating concentrations of cortisol, albeit the difference was not significant **(12)**. However, whether mild energy restriction – as is commonly used in long-term lifestyle management of overweight and obesity – also stimulates cortisol output or hypothalamo-pituitary-adrenal function is less clear. One study in obese people showed no effect of moderate energy restriction (30% kJ restriction) for 12 weeks resulting in a weight loss of about 8 kg on 24-h urinary free cortisol/cortisone, nor on basal- or ACTH-stimulated free and total cortisol, or corticosteroid-binding globulin levels in the circulation **(85)**. In an independent study, however, the increased appetite measured in overweight people after a weight loss programme consisting of moderate energy restriction was significantly correlated with fasting plasma cortisol levels, which was the most consistent predictor of the associated increase in appetite **(9)**. As glucocorticoids such as cortisol can promote the preservation or accumulation of adipose tissue **(86–91)** as will be discussed below (see Effects of activation of the hypothalamo-pituitary-adrenal axis on body weight and composition), increased cortisol output – if present – could conceivably contribute to the conservation of fat stores during weight loss attempts by dietary modification and physical activity.

Effects of hypothalamic regulators of energy balance on the adrenal axis

The changes that occur in the hypothalamus after energy restriction are likely to contribute to the associated increases in activity of the hypothalamo-pituitary-adrenal axis. As summarized in Fig. 1, acute increases in NPY-ergic activity in the hypothalamus *in vivo* increase CRH expression and secretion in the hypothalamic PVN, and increase circulating concentrations of ACTH and corticosterone **(92,93)**. This effect probably occurs via direct NPY-ergic innervation of CRH-synthesizing neurons in the PVN **(92,94)** and not via direct action of NPY on corticotroph cells in the anterior pituitary **(95)**. Pharmacological and knockout studies show that several of the five known Y receptors mentioned in the Introduction mediate NPY's stimulatory effects on the adrenal axis. Indeed, deletion of Y2 or Y4 receptors completely attenuates and Y1 receptor deletion partially attenuates the high circulating concentrations of corticosterone in male genetically obese leptin-deficient *ob/ob* mice **(96–98)**, which exhibit chronically elevated hypothalamic expression of NPY **(99)**. In addition to effects via Y1, Y2 and Y4 receptors, ICV administration of a Y5 receptor antagonist almost completely blocks the stimulatory effect of ICV-administered pancreatic polypeptide, an endogenous gut-derived Y-receptor agonist and member of the NPY family of peptides, on circulating ACTH and corticosterone concentrations in conscious male rats **(100)**. Therefore, NPY and related peptides such as pancreatic polypeptide can induce effects on the hypothalamo-pituitary-adrenal axis via a variety of Y receptors.

In addition to stimulatory effects induced by NPY or Y receptor activation, the adrenal axis may also be regulated by the melanocortin system, albeit only transiently. CRH-expressing neurons in the PVN were reported to express MC4 receptors, and acute ICV or intra-PVN administration of the melanocortin agonist melanotan-II (MTII) or an α-MSH analogue to male

rats increased CRH mRNA expression in the PVN within 15 min and increased plasma ACTH and corticosterone concentrations within 10 min **(101,102)**. Corticosteronemia peaked at 30 min and was normalized by 120 min of ICV MTII injection, and the effect was attenuated by blockade of MC4 or CRH receptors **(101)**, demonstrating MC4 receptor-mediated effects on the CRH axis. The physiological significance of this finding is unclear, however, given that acute central administration of the melanocortin receptor antagonists HS014 or AgRP has the same transient stimulatory effect on corticosteronemia **(103)** or plasma ACTH **(102)** as the agonists MTII **(101)** or an α-MSH analogue **(102)**. Additionally, male and female MC4 receptor knockout mice do not have perturbations in plasma corticosterone concentrations **(104)**. Moreover, whereas chronic central infusion of an MC4 receptor antagonist increases corticosteronemia in male rodents, no such change is seen when animals are pair-fed with controls to prevent hyperphagia **(103,105)**, demonstrating that the stimulatory effect on corticosteronemia was likely mediated by hyperphagia. Thus, while changes in melanocortin signalling may induce short-term stimulatory or inhibitory effects on CRH expression or circulating corticosterone levels, they do not appear to exert long-term regulatory effects on the adrenal axis that would contribute to the changes observed in this axis with energy restriction.

Hypothalamic CART expression is decreased with negative energy balance or in leptin-deficient obesity **(106)**, it inhibits food intake when given ICV but stimulates food intake when microinjected directly into the hypothalamus **(32)**, and it stimulates factors that would tend to increase metabolic rate (such as expression of uncoupling proteins and thyroid function) **(71,107–109)**. Therefore, CART has some effects that are similar to those of anorexigenic agents such as α-MSH. On the other hand, it is likely that CART, like the orexigenic NPY, is an activator of the adrenal axis, because it increases CRH secretion from hypothalamic explants *in vitro*, and ICV or intrahypothalamic administration of CART to normal male rats (at doses that result in reduced and increased food intake, respectively), both resulted in increased plasma concentrations of ACTH or corticosterone **(72,110)**, albeit the effect on circulating ACTH levels was seen only after 30 min and not after 60 min **(111)**. CART can therefore be seen to have some effects (such as hypophagia, stimulation of the thyroid axis and hypermetabolism) that would promote weight loss, and other effects (notably hyperphagia and stimulation of the adrenal axis) that could promote fat accumulation. As such, CART may play a dual role in the regulation of energy homeostasis.

Inhibition of the hypothalamo-pituitary-gonadal axis

Situations of negative energy balance – such as famines or heavy exercise – are associated with reduced or abolished reproductive functions in animals and humans **(112)**. This phenomenon helps to conserve energy and is thought to promote species survival during times of insufficient food intake. Reproductive functions in mammals are regulated by the hypothalamo-pituitary-gonadal axis. Gonadotropin-releasing hormone (GnRH), a central neuropeptide expressed in the medial pre-optic area (MPA) and medial septal nucleus **(113–115)**, regulates the synthesis and release of the anterior pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which subsequently act on the gonads that produce sex hormones. The reproductive axis has the capacity to respond to changes in nutritional status, and the sensitivity of the reproductive axis to current energy availability has been demonstrated by the fact that even subtle declines in energy availability can produce clinically recognized menstrual disturbances in women **(116)**. Studies in rodents and humans suggest that the inhibition of reproductive function during negative energy balance occurs via inhibition of the entire hypothalamo-pituitary-gonadal axis, as outlined below and as schematized in Fig. 1.

Changes in gonadal axis function with energy restriction in animals

Adverse metabolic conditions are associated with reduced or abolished reproductive functions in rodents. Food deprivation or chronic energy restriction caused decreased ovarian weight, prolonged dioestrus, and delayed sexual maturation in female rodents **(34,117)** and a significant reduction in serum testosterone levels in male rodents **(34,118,119)**. At the hypothalamo-pituitary level, energy deficit leads to marked reductions in serum gonadotropin levels **(118–120)** and decreases in hypothalamic GnRH mRNA expression **(119,121)**. Furthermore, the starvation-induced impairment in gonadotropin and testosterone secretion can be reversed by pulsatile administration of GnRH **(122)**, highlighting the crucial role of hypothalamic GnRH in the suppressive effect of negative balance on reproductive function, at least in males, in which most

of these studies have been conducted.

Changes in gonadal axis function with energy restriction in humans
In lean adults, energy restriction is associated with diminished activity of the reproductive axis. For instance, lean young men undergoing 8 weeks of US Army Ranger Training (consisting of heavy exercise and food restriction with marked weight loss) showed significant reductions in serum concentrations of LH and testosterone with a concomitant increase in circulating sex hormone-binding globulin levels **(83)**. The latter binds testosterone and reduces the circulating concentrations of free testosterone, which is thought to be more bioavailable than bound testosterone, at least in certain tissues **(123)**. Similar inhibition of the gonadal axis, as indicated by significantly reduced circulating concentrations of progesterone or reduced frequency of LH pulses, has been observed in lean women during seasons of heavy physical activity associated with weight loss **(124)**, or in healthy lean young women in whom energy availability was experimentally reduced for 5 d **(125)**. Anorexia nervosa presents with central inhibition of the gonadotropic axis, as indicated by reductions in 24-h gonadotropin secretion and absence of the normal pulsatile peaks in circulating LH levels, as well as low circulating testosterone and oestradiol levels **(45)**. Additionally, anorexia nervosa and high volumes of physical activity (for example, in female dancers, runners and divers) are frequently associated with amenorrhea **(126,127)**, in keeping with inhibition of reproductive functions under conditions of relative energy deficit.

Some (but not all) studies indicate that energy restriction and weight loss in overweight or obese people may also inhibit the hypothalamo-pituitary-gonadal axis, at least when measured immediately after weight loss. These studies are listed in Table 1. Overweight men who lost weight by 25 d on a very low energy diet showed significant reductions in serum concentrations of prolactin, LH and free testosterone, a tendency towards reduced FSH, no change in total testosterone and a significant increase in sex hormone-binding globulin **(12)**. Similarly, overweight or obese men who lost at least 4% of their body weight during 10 d of total fasting showed blunted FSH responses to GnRH stimulation, significantly reduced serum concentrations of FSH and testosterone, with significantly increased urinary excretion of LH and FSH **(128)**. In contrast, however, some studies have shown that weight loss in overweight or obese men is associated with increases or no change in circulating total or free testosterone levels, as recently reviewed **(123)**. For instance, in obese men with mild hypoandrogenism, 9 weeks on a very low energy diet resulted in a marked increase in circulating sex hormone-binding globulin levels with significant increases in serum concentrations of free and total testosterone, with effects persisting during a subsequent 12-month maintenance period **(129)**. To our knowledge, there are no studies investigating effects of weight loss in overweight or obese women on function of the gonadotropic axis, nor are there any studies comparing effects of different degrees of energy restriction on sex hormone levels in men or women. As declines in androgens and oestrogen production can contribute to fat accumulation and loss of lean mass as will be discussed below (see Effects of inhibition of the hypothalamo-pituitary-gonadal axis on body weight and composition), changes in hypothalamo-pituitary-gonadal function during weight loss interventions – if present – could be an important factor influencing body composition.

Effects of hypothalamic regulators of energy balance on the gonadal axis
As outlined in Fig. 1, energy deficit-induced inhibition of the reproductive axis has been hypothesized to be at least in part due to the associated increase in hypothalamic NPY expression, because ICV NPY administration recapitulates the hypogonadism induced by fasting **(117,130–134)**. Seven-day administration of NPY to the lateral cerebral ventricle via osmotic minipumps significantly reduced pituitary weight, seminal vesicle and testis weights, and circulating testosterone levels in intact male rats **(132)**, and delayed sexual maturation, disrupted estrous cyclicity and reduced the number of pituitary GnRH receptors in normal female rats **(117,131,134)**. The fasting-induced suppression of LH levels is attenuated when the associated up-regulation of hypothalamic NPY expression is inhibited by ICV administration of leptin or ciliary neurotropic factor **(135)**. Furthermore, *ob/ob* mice of both sexes, which exhibit elevated hypothalamic expression of NPY and are hypogonadal and infertile, show marked improvement in gonadotropic function and partial restoration of fertility when crossed with NPY-deficient mice **(136)**, providing additional evidence that elevated hypothalamic NPY expression causes repression of gonadal function. These effects of NPY may be due to inhibition of the release of GnRH **(122,137,138)**. Indeed, NPY fibres are in close proximity to the dendrites

and cell bodies of GnRH neurons in the MPA **(139)**, and NPY fibres in the median eminence may also act on GnRH terminals **(140)**. Of the five known Y receptors, NPY-mediated repression of the gonadal axis likely occurs through Y4 receptor agonism, because fasting-induced reductions in GnRH mRNA expression in the MPA as well as the associated reductions in testis testosterone content were abolished by germ line knockout of Y4 but not Y2 receptors **(121)**. Moreover, Y4 receptor knockout rescues the hypogonadism and infertility of male and female *ob/ob* mice **(98)**, highlighting the critical role of Y4 in regulating reproductive processes under conditions of chronically elevated central NPY-ergic activity, such as in leptin deficiency or chronic energy restriction.

In addition to effects of NPY, a role of POMC/CART neurons in conveying metabolic status to GnRH neurons has been suggested by some neuroanatomy studies. POMC/CART-producing neurons in the arcuate nucleus project to the MPA, and nerve terminals containing POMC products (β-endorphin and α-MSH) and CART make apparent synaptic contact with GnRH-immunoreactive cells **(141,142)**. Interestingly, differential effects of the various POMC gene products on the reproductive axis have been observed, with stimulation of sexual activity by α-MSH **(143,144)**, but inhibition of GnRH and LH secretion by β-endorphin **(145–147)**. However, deletion of POMC **(148)** or the MC4 receptor **(104)**, a brain target through which α-MSH induces anorexia, did not affect fertility in mice of either sex, suggesting that the melanocortin system may not be essential for reproduction. In line with these observations, 7-d chronic ICV infusion of the MC3/4 receptor antagonist SHU9119, which pharmacologically mimics knockout of the MC3 and MC4 receptor subtypes, generated a distinct obesity syndrome without altering the function of the gonadotropic axis in male mice **(149)**. Similarly, administration of the synthetic MC receptor agonist MTII to male *ob/ob* mice did not rescue the impaired reproductive function of these mice **(150)**. ICV but not intrahypothalamic (PVN) administration of CART to male rats, at a dose that significantly inhibits food intake, has been shown to significantly increase plasma prolactin concentrations **(72)**, but further work is needed to determine whether CART regulates various reproductive functions under differential conditions of energy availability. Taken together, these studies show that NPY and the Y4 receptor are likely to be physiological regulators of the gonadotropic axis and reproductive functions under conditions of negative energy balance, but that the melanocortin system – while possibly modulating the function of this axis under certain circumstances – may not be a long-term regulator of reproduction under conditions of long-term energy deficit.

Inhibition of the hypothalamo-pituitary-somatotropic axis or insulin-like growth factor-1

The hypothalamo-pituitary-somatotropic axis is crucial for normal development, somatic growth and other metabolic functions. In the hypothalamus, neurons expressing growth hormone-releasing hormone (GHRH) are localized in the arcuate and ventromedial hypothalamic nuclei **(151)**, and exert a stimulatory effect on the production and release of growth hormone (GH) from the pituitary gland. Circulating GH acts on many tissues, notably the liver, to stimulate the production of IGF-1, the essential mediator of most of GH's actions on growth **(152)**. IGF-1 also inhibits GH synthesis and secretion by direct effects on both the pituitary gland and the hypothalamus in a short-loop feedback **(153–155)**. As synthesized in Fig. 1, energy deficit has been shown to have divergent effects on the hypothalamo-pituitary-somatotropic axis in rats, mice and humans, but all species share a common effect of energy deficit: reduction in the circulating concentrations of IGF-1.

Changes in somatotropic axis function with energy restriction in rodents

Hypothalamic GHRH mRNA expression was recently shown to have a biphasic response to fasting in male mice, with a significant increase during the first 24 h of fasting followed by a 50% decrease at 48 h after food withdrawal **(156)**. Longer periods of food deprivation (48–72 h) are consistently reported to reduce GHRH expression in the hypothalamus of male rats and mice **(156–159)**. The effects of food deprivation on GH secretion from the pituitary gland are species dependent. In rats, fasting progressively suppressed GH pulse amplitude, decreased GH secretion and reduced circulating GH concentrations **(157,158,160–165)**. In mice, however, circulating GH levels rise in response to food deprivation **(156,159,166)**. Despite the differential effects of food deprivation on GH release in rats and mice, fasting increases pituitary GHRH sensitivity in both species, as indicated by increases in pituitary GHRH receptor mRNA and protein levels as well as an increase in GHRH binding in the pituitary **(156,158,167)**. Fasting also consistently

decreases concentrations of the final effector, IGF-1, in the circulation in both rats and mice **(157,158,168–171)**. A decrease in hepatic sensitivity to GH stimulation of IGF-1 production is thought to contribute to the fall in IGF-1 levels in fasted rodents **(172)**. At least some of the effects of energy restriction on the GH axis are likely to be mediated via the fasting-induced induction of hepatic fibroblast growth factor 21 (FGF21) **(173)**. In keeping with this, transgenic mice over expressing FGF21 have reduced growth in association with increased circulating concentrations of GH and reduced circulating IGF-1 levels, indicating GH resistance **(173)**. FGF21 may mediate these effects by direct action on hepatocytes, where it strongly impairs activity of signal transducer and activator of transcription 5, an important mediator of GH actions, and induces expression of IGF-1 binding protein 1 (IGFBP1) and inhibits that of suppressor of cytokine signalling 2, both changes contributing to blunting of GH signalling **(173)**. Taken together, negative energy balance has been shown to have multiple and divergent effects on the function of the somatotropic axis in rats and mice, at least in males in which the majority of these studies have been conducted, but both species share an ultimate inhibition of the circulating concentration of the main effector, IGF-1.

Changes in somatotropic axis function energy restriction in humans
In humans, as in mice, energy deficit is known to increase circulating concentrations of GH with a concomitant decrease in circulating levels of IGF-1 **(174)**. This effect is not restricted to lean people; it is also seen in overweight individuals. The significant weight losses seen in lean young men during US Army Ranger Training, as well as in overweight men on a very low energy diet, are associated with marked increases in circulating GH levels or reductions in the circulating concentrations of IGF-1 (Table 1) **(12,83)**. As reduced serum IGF-1 levels can increase body fat, reduce bone mineral density **(175)** and probably play a causative role in the loss of lean tissues with ageing **(176)** as will be discussed below (see Effects of inhibition of the hypothalamo-pituitary-somatotropic axis on body weight and composition), reduced circulating IGF-1 levels could conceivably contribute to the preservation of body fat and loss of lean mass seen during weight loss.

Effects of hypothalamic regulators of energy balance on the somatotropic axis
The fasting-induced reduction in hypothalamic GHRH expression that has been observed in male rats and non-human primate has been proposed to be due to the concomitant increase in hypothalamic NPY expression (Fig. 1) **(177–179)**. In keeping with this, ICV administration of NPY to rats and mice profoundly inhibits the somatotropic axis by reducing hypothalamic GHRH expression **(130)**, reducing the pituitary content of GH (female rats) **(131)**, abolishing the normal pulsatile release of GH into the circulation **(132)**, and consequently reducing the plasma concentrations of GH and its main effector in the periphery, IGF-1. The importance of NPY in mediating the fasting-induced reduction in hypothalamic GHRH mRNA expression was demonstrated in male NPY knockout mice, which, unlike wild-type mice, showed no fasting-induced drop in hypothalamic GHRH expression **(159)**. NPY's effects on the somatotropic axis are likely mediated by hypothalamic Y2 receptors, because germline or hypothalamus-specific Y2 (but not Y4) receptor ablation in male mice abolishes the fasting-induced reductions in hypothalamic GHRH mRNA expression and serum IGF-1 levels **(121)**.

In contrast to the clearly demonstrated role of NPY, changes in hypothalamic expression of POMC and the melanocortin system in response to fasting may not play an important role in mediating the effects of energy deficit on the somatotropic axis, because neither activation of MC3/4 receptors by the MC3/4 agonist MTII **(180)** nor deactivation of MC3/4 receptors by the antagonist SHU9119 **(149)** had any effects on functions of the somatotropic axis in male rats. While CART has been shown to significantly increase plasma concentrations of GH after ICV injection of a dose that significantly reduces food intake in male rats, it had no significant effect on plasma GH levels after microinjection into the PVN **(72)**. It is therefore unclear whether or how CART plays a role in regulation of the somatotropic axis under different conditions of energy availability.

Impact of neuroendocrine alterations on body weight and composition

From the above considerations it can be seen that short- or long-term energy deficit in lean or obese rodents or humans generally inhibits function of the hypothalamo-pituitary-thyroid axis, may stimulate activity of the hypothalamo-pituitary-adrenal axis as well as inhibiting the

hypothalamo-pituitary-gonadotropic axis and circulating concentrations of IGF-1. The resultant changes in circulating concentrations of thyroid hormones, glucocorticoids, sex hormones and IGF-1 during energy deficit could have profound effects on body weight, body composition and fat distribution as discussed below and as summarized in Fig. 1.

Effects of inhibition of the hypothalamo-pituitary-thyroid axis on body weight and composition

Thyroid hormones play a well-known role in regulating energy expenditure. In the complete absence of thyroid hormones, basal metabolic rate or resting energy expenditure is reduced by up to 30%, indicating that as much as 30% of basal metabolic rate is thyroid hormone dependent **(181)**. Resting energy expenditure is also remarkably responsive to variations in thyroid hormone levels around the euthyroid state **(182)**, and significant changes in resting energy expenditure occur in response to spontaneous fluctuations in circulating free T4 concentration in normal lean men **(183)**. In hypothyroid patients, oxygen consumption, heat production and basal metabolic rate are significantly reduced relative to euthyroid controls **(184)**. In addition to their role in maintaining resting energy expenditure, thyroid hormones are important for adaptive thermogenesis. Hypothyroid patients are characteristically cold intolerant **(185)**, and this is recapitulated in hypothyroid animals, which are unable to produce sufficient heat to maintain body temperature and prevent hypothermia in a cold environment **(186,187)**. Evidence suggests that thyroid hormones are also involved in diet-induced thermogenesis and adaptive changes in energy expenditure in response to excess food consumption **(188)**, because hypothyroid rats were unable to increase energy expenditure and show a normal thermic effect of food in response to high-fat feeding **(188)**, as has been observed in euthyroid **(189–192)** and hyperthyroid rats **(193)**. Thyroid hormones regulate energy expenditure via direct actions on peripheral tissues to influence cellular metabolism **(181)**, as well as via actions on hypothalamic sites to regulate adenosine monophosphate-activated protein kinase, fatty acid metabolism and subsequent sympathetic nervous output **(194)**. Because of their prominent effects on energy expenditure, involuntary changes in body weight are clinical indicators of abnormal thyroid function, often prompting testing of thyroid function **(181)**. Severe hyperthyroidism causes noticeable weight loss, albeit milder hyperthyroidism is not always associated with weight loss, likely due to compensatory hyperphagia as described below **(181)**. On the other hand, cross-sectional studies show that hypothyroidism is sometimes associated with slight increases in body weight or body mass index (BMI) **(181,195–197)**.

In addition to effects on energy expenditure and body weight, thyroid hormones have effects on food intake. The hyperthyroid state is associated with increased food intake in humans **(181)** and in experimental animals **(198–201)**, whereas hypothyroidism is associated with reduced food intake **(201)**. Hyperthyroidism may promote hyperphagia via actions in the hypothalamus, because it has been shown that T3 can stimulate orexigenic NPY/AgRP neurons by enhancing uncoupling protein 2 expression and subsequent mitochondrial proliferation **(43)**. The increased appetite of hyperthyroidism may also be secondary to the associated state of negative energy balance, and its importance in preventing additional weight loss is demonstrated in the accelerated weight loss observed when food intake is limited in hyperthyroidism **(202)**. Body weight remains relatively stable during the transition from hypothyroidism to euthyroidism during thyroid hormone treatment, and this is likely due to the increased appetite associated with thyroid hormone treatment **(196,197)**.

Free fatty acids derived from adipose tissue are the primary source of substrate for the increased energy expenditure due to elevated thyroid hormone levels, and the 15–19% increase in energy intake in hyperthyroidism is channelled through lipogenesis to help maintain fat stores **(200,201)**. In keeping with this, T3 stimulates fatty acid oxidation **(203)**, enhances lipolysis by increasing the sensitivity of the process to catecholamines **(203)**, as well as increasing the expression of genes encoding lipogenic enzymes **(204–208)**. Despite the concomitant increase in lipogenesis, the increased fat oxidation in hyperthyroidism results in significant net reductions in fat mass in humans **(181,209)** and in experimental male rats **(200,201)**.

Whereas hyperthyroidism reduces fat mass in rodents and humans, hypothyroid states are associated with significant increases in lipid accumulation. This is consistent with the decreased fatty acid oxidation observed in hypothyroidism **(210)**. Interestingly, hypothyroidism has also been shown to decrease total body protein content in male rats **(201)**. This may be due to the reduced protein synthesis characteristic of hypothyroid states **(210)**, as well as to an increased utilization of amino acids as fuels when lipid utilization is limited, suggesting a shift

from lipids to amino acids as oxidative fuels in the hypothyroid state **(201)**. In addition to effects on lipid and protein metabolism, a recent study has demonstrated a role of thyroid hormones in the regulation of bone homeostasis, with hypothyroidism impairing longitudinal growth and gain in bone mineral density, and these effects are partially reversed by a synthetic analogue of thyroid hormones **(211)**.

Taken together, thyroid hormones have important roles in the regulation of energy expenditure, appetite, lipid and protein metabolism as well as bone homeostasis. Hypothyroidism, or inhibition of the hypothalamo-pituitary-thyrotropic axis, leads to a modest propensity for greater weight gain as well as metabolic changes that would be expected to shift body composition towards an increase in fat mass in association with a reduction in lean mass and possibly also bone mass. It is therefore possible that the inhibition of thyroid function induced by long-term energy deficit (as in long-term lifestyle interventions for weight loss in overweight or obese individuals) could have a negative influence on the propensity for weight regain and on body composition.

Effects of activation of the hypothalamo-pituitary-adrenal axis on body weight and composition

Activation of the adrenal axis leading to increases in circulating levels of glucocorticoids would be expected to promote fat accretion at the expense of lean tissues and bone, because glucocorticoids *per se* can cause marked changes in body composition in non-obese animals and humans. Lean men and women taking a high dose of oral glucocorticoid treatment (≥40 mg d−1 of a prednisolone equivalent) for 2 months showed a 10% increase in fat mass, a 10% decrease in lean body mass, and significant decreases in bone mineral density and bone mineral content in the absence of effects on body weight **(212)**. Longer-term (more than 60 d) use of oral glucocorticoids is associated with self-reported weight gain in over 60% of patients, including those on lower doses (e.g. 10 mg d−1 prednisone for 6 months), and weight gain is the most commonly reported adverse event in patients taking glucocorticoids **(213)**. Additionally, people with Cushing's syndrome, associated with primary hypercortisolism, exhibit hyperphagia, weight gain, visceral obesity and muscle wasting **(214)**, further demonstrating a primary role of increased glucocorticoid action in the propensity to store fat (particularly central fat) at the expense of lean tissues.

Animal studies provide insights into mechanisms by which glucocorticoids induce weight gain. Peripheral corticosterone administration dose dependently increased food intake, fat depot weight, adipocyte size, lipoprotein lipase activity and insulinemia of normal or adrenalectomized rats while decreasing food efficiency **(87,88)**. These effects are likely mediated via the brain, because another study using lower doses found that ICV but not subcutaneous corticosterone injection increased weight gain of adrenalectomized rats **(89)**. Furthermore, 3-d low-dose ICV dexamethasone infusion in normal rats increased food intake, body weight gain, plasma triglyceride, leptin, and insulin levels, and decreased brown adipose tissue thermogenin expression **(90)**. When given intraperitoneally, the same dose of dexamethasone decreased food intake and body weight gain, and had much less marked effects on leptinemia and insulinemia **(90)**, in keeping with central mechanisms of action.

In light of these findings, it is possible that the increased circulating levels of glucocorticoids that have been observed during energy restriction in animals and humans, particularly during severe energy restriction, act centrally to promote fat conservation – notably central fat – at the expense of lean body mass and bone.

Effects of inhibition of the hypothalamo-pituitary-gonadal axis on body weight and composition

Given that sex hormones exert significant effects on body composition and mechanisms that control energy homeostasis, it might be expected that the decreased function of the hypothalamo-pituitary-gonadal axis and circulating sex hormone levels that have been observed during energy restriction could also have significant consequences on body weight and body composition.

Oestrogen deficiency in female animals is clearly associated with transient hyperphagia and increased body weight and adiposity, especially visceral adiposity **(215–217)**. In women, some studies (although not all) report that menopause is associated with weight gain and loss of fat-free mass independent of age, and the majority of studies observed increased abdominal or visceral adiposity at menopause as recently reviewed **(218,219)**. Several studies have shown a reduction in levels of physical activity in women going through the menopausal transition

(219,220) and in rodents after ovariectomy **(221)**, and this likely contributes to the associated decreases in fat-free mass, energy expenditure or metabolic rate as well as increases in adiposity **(219–221)**. Further to its effects on adiposity and fat-free mass, oestrogen has numerous effects on bone – some via direct tissue actions – to stimulate bone formation and decrease bone resorption as recently reviewed **(222)**. Lack of gonadal oestrogens contributes to the markedly greater propensity for osteoporosis in post-menopausal vs. pre-menopausal women **(222)**. In both ovariectomized animals and oestrogen-deficient women, administration of exogenous oestrogens has been shown to reduce body weight, prevent abdominal or visceral fat gain and reduce loss of fat-free mass, muscle strength and bone mass **(216,222,223)**, demonstrating the pivotal role of oestrogens and lack thereof in mediating significant alterations in body weight and body composition.

In addition to oestrogens, androgens also impact body composition. In men, the observed gradual decline in circulating androgens with ageing (the so-called 'andropause') is accompanied by increased total and abdominal fat **(224)**. Several studies have shown that administration of aromatizable androgens such as testosterone in older men reduces both total and abdominal fat and increases lean mass **(225–228)**. Declines in oestrogen and androgen production are likely to play a role in the pathogenesis of sarcopenia (age-related decline in muscle mass and quality) and ageing-induced increases in fat mass **(176)**. Androgens additionally 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 post-menopausal period in females, as well as in ageing males, is crucial for the development of osteoporosis **(222)**. It remains to be determined to what extent the decrease in sex hormone levels that occur with energy restriction contributes to loss in muscle mass and conservation of fat, and whether attenuation of such reductions in circulating sex hormone levels may improve fat loss and prevent loss of fat-free mass or bone during weight loss interventions in overweight and obese individuals.

Effects of inhibition of the hypothalamo-pituitary-somatotropic axis on body weight and composition

The reduction in circulating IGF-1 concentrations that have been observed in response to energy deficit could contribute to an associated tendency to conserve fat mass and lose lean mass and bone, because experimentally manipulating circulating IGF-1 levels has significant effects on body composition as recently reviewed **(229)**. IGF-1 is known as the main mediator of the well-known anabolic effects of GH on lean tissue and bone **(152,229)**, but it also has effects on fat metabolism. Female congenic mice with a 20% reduction in circulating IGF-1 levels show significantly increased body fat and reduced bone mineral density **(175)**. Additionally, declines in the local action of IGF-1 in muscle and reduced serum IGF-1 levels with age are implicated in sarcopenia (age-related decline in muscle mass and quality) **(176,230)**. In keeping with these findings on the effects of reduced IGF-1, when exogenous IGF-1 was administered to GH-deficient people for 8 weeks, it led to a significant decrease in fat mass and a significant increase in lean mass in association with increased lipolysis and lipid oxidation rates, albeit longer-term (1 year) treatment with IGF-1 in healthy post-menopausal women was not associated with any changes in lean body mass or adiposity **(231)**.

It remains to be determined the extent to which any drop in circulating IGF-1 levels with weight loss in overweight or obese people may contribute to fat conservation and loss of lean body mass or bone during energy restriction, and whether preventing this drop will improve fat loss and attenuate loss of lean tissue or bone during long-term dietary interventions for weight loss. It is particularly noteworthy that addition of resistance exercise, but not aerobic exercise, to a diet-induced weight loss programme resulting in an approximately 12 kg weight loss, completely prevented the loss of lean body mass and muscular strength that was seen in the no-exercise control group **(232)**, and 13–25 weeks of resistance training in middle-aged men and women has been shown to increase circulating concentrations of IGF-1 and reduce that of the binding protein IGFBP3 **(233)**. Another study in older men and women showed no effect of a 24-week resistance training programme on circulating IGF-1 and IGFBP3 levels **(234)**, but IGF-1 is produced by myocytes in addition to hepatocytes, and resistance-exercise training-induced effects on intramuscular IGF-1 content **(235)** cannot be excluded. In brief, these findings are supportive of a possible role of IGF-1 in the maintenance of muscle mass during weight loss interventions, and further investigation into the effects of different methods of weight loss on

IGF-1 could help to identify those methods that prevent loss of lean body tissues.

Other hormonal effects of energy restriction and their potential consequences on body composition

In addition to changes in activities of the neurohormonal axes discussed in this review (the thyrotropic, corticotropic, gonadotropic and somatotropic axes), negative energy balance affects many other hormone systems that regulate body composition. For instance, adaptive changes in gut function in response to negative energy balance include increased circulating concentrations of the 'hunger hormone' ghrelin **(236,237)**, and decreased circulating concentrations of the 'satiety hormone' peptide YY **(237,238)**, which belongs to the NPY family of peptides and acts on Y receptors. These changes could contribute to increased appetite **(239–241)** as well as changes in body composition, as indicated by effects of ghrelin on anterior pituitary function **(242)** and by the effects of peptide YY knockout and transgenic overexpression on the somatotropic axis and lean mass in mice **(243,244)**.

Implications for ongoing obesity research

Changes in body composition in response to energy deficit

In light of the above hormonal effects of energy deficit and their possible impact on body composition, it is conceivable that weight loss in overweight and obese individuals may not induce purely positive body compositional changes. As an extreme example, elite athletes in negative energy balance **(245)** and people with anorexia nervosa **(45)** exhibit reduced bone mineral density or reductions in indices of bone formation and parallel increases in bone resorption indices. These skeletal effects of extreme energy deficit could be due to the associated hormonal changes described throughout this review, as well as to the lack of nutrients available for construction and maintenance of skeletal tissue **(45)**. It is currently unclear whether the low bone mass induced by anorexia nervosa is fully reversed with refeeding **(45)**. As another example of adverse sequelae of extreme energy deficit, adult women with anorexia nervosa – immediately upon restoration of normal body weight – showed increases in visceral fat mass compared with weight-matched control women, albeit this propensity for increased central obesity was no longer observed after 1 year of maintenance of normal body weight **(246)**. Whether weight loss in overweight or obese individuals induces similar adverse body compositional changes, and whether any such changes persist in the long term, remains to be determined.

Assessment of body compositional changes in weight loss trials

In order to unambiguously determine whether various weight loss interventions induce deterioration of body composition in overweight or obese people, future clinical trials will need to use more sensitive measures of body composition than anthropometry and dual energy x-ray absorptiometry (DXA). The reason for this is exemplified in a study comparing effects of exercise on weight regain at 12 months after ~5 months on a very low energy diet **(247)**. In this study, abdominal magnetic resonance imaging (MRI) revealed significant differences in deep subcutaneous adipose tissue regain (54% for aerobic vs. 9% for resistance exercise, n = 18–21), with no significant differences in regain of body weight, BMI, DXA-derived percent fat, waist circumference or DXA-derived trunk fat **(247)**. Similarly, thigh muscle mass determined by MRI showed significant differences between weight loss interventions differing in protein content (n = 13), whereas lean soft tissue mass determined by DXA was not significantly different between interventions **(248)**. Further, after a 16-week exercise programme, healthy older women exhibited significant decreases in visceral fat (−9.7%) and mid-thigh subcutaneous adipose tissue (−5.7%), and significant increases in mid-thigh muscle (9.6%) as determined by computerized tomography (CT), despite no changes in body weight or DXA-derived percent fat or fat-free mass **(249)**. As such, techniques such as MRI and CT, while considerably more expensive than DXA or anthropometric measures, provide greater insights into effects of different weight management regimes on clinically relevant regional changes in body composition.

Very low energy diets

The neuroendocrine effects of energy deficit raise questions as to the potential impact on body composition of weight loss via severe energy restriction (i.e. very low energy diet), which is increasingly being used for the clinical management of obesity **(250)**. While very low energy diets significantly blunt the increased appetite normally associated with weight loss, possibly due to generation of ketone bodies **(251)**, some studies suggest that such severe energy restriction may induce stronger adaptive responses to weight loss than moderate energy restriction. For instance, head-to-head comparisons show that severe energy restriction leads to greater reductions in weight loss efficiency (body energy loss divided by energy deficit) **(252)**, greater declines in physical activity **(2)** and greater losses of lean body mass **(253)** than moderate energy restriction. To our knowledge, no studies have directly compared the effects of severe vs. moderate energy restriction on neuroendocrine adaptations. If weight loss via very low energy diets does indeed induce stronger adaptive changes in neuroendocrine status, it is conceivable that the current clinical resurgence of modern very low energy diets may be promoting a hormonal milieu that increases the risk of long-term adverse consequences such as central obesity and sarcopenia.

Gastric surgery for obesity

An additional question for ongoing research is the effects of gastric surgery on the adaptive responses to weight loss. Roux-en-Y gastric bypass surgery is the single most effective treatment for obesity currently available, with loss of 49–62% of excess weight being maintained at 10–14 years following surgery **(254–256)**. Laparoscopic sleeve gastrectomy has recently emerged as being almost as effective as gastric bypass surgery, and both surgical techniques are more effective at inducing weight loss than laparoscopic vertical banded gastroplasty ('gastric banding') **(237)**. While both sleeve gastrectomy and gastric bypass surgery have been shown to dramatically reduce measures of hunger, desire to eat as well as actual food intake **(237)**, evidence suggests that the success of these two surgeries is additionally due to hormonal and metabolic effects that attenuate the normal adaptive response to energy restriction and weight loss. For instance, whereas weight loss by energy restriction typically plateaus within a few months – indicative of energy-conserving effects – patients with Roux-en-Y gastric bypass continue to lose weight without major plateaus until about 1 year post-surgery **(256)**. Moreover, obese patients who had gastric bypass surgery showed smaller decreases in resting energy expenditure than would have been expected with similar weight reduction by comparable energy restriction, albeit no direct comparison with very low energy diets was made in that study **(257)**. Further evidence for gastric surgery-induced attenuation of the adaptive responses to energy restriction comes from the finding that while weight loss induced by dietary restriction is associated with increased circulating levels of the orexigenic hormone, ghrelin **(236,237)**, and decreased circulating concentrations of the anorexigenic hormone, peptide YY **(238)**, these changes are either absent or reversed in patients with gastric bypass or sleeve gastrectomy, perhaps contributing to the associated decrease in appetite **(237)**. These observations raise the possibility that other adaptations to energy deficit – such as the neuroendocrine changes detailed in this review – may also be abated by gastric bypass or sleeve gastrectomy. If so, then the associated changes in body composition might be expected to be more favourable than those induced by gastric banding (which is not associated with as marked an attenuation of appetite or gut hormone responses **(237)**), or by lifestyle-induced energy restriction. Consistent with this possibility, people who underwent gastric bypass surgery showed significantly less loss of bone mineral content and bone mineral density, and significantly greater visceral adipose tissue loss, in the first year after surgery than people who had undergone gastric banding **(258)**. These changes – as well as the lack of difference in lean tissue mass between the groups – are all the more noteworthy given that gastric bypass patients lost significantly more weight and total body fat than those who underwent gastric banding. Future comparative studies of obesity surgery could benefit from blood sampling for determination of neuroendocrine status, as this may provide explanations for such clinically relevant differences in body composition.

Summary and conclusions

Negative energy balance is known to increase hunger and to decrease metabolic rate, and it may

also reduce the propensity for physical activity and the energy cost of activity **(1–4,6,7)**. These changes have been measured in laboratories around the world, and members of the public have noted them in their personal experiences of lifestyle-induced weight reduction, as indicated by increased desires to eat, cold intolerance, decelerated or stalled weight loss and lethargy. Possibly more insidious than these changes, however, are the adaptations that have been *infrequently* assessed in clinical weight loss trials and that are not consciously noticed by end users, but which have the potential to produce long-term adverse consequences on body composition. Such changes include reduced thyroid function, increased adrenal function, and decreased gonadotropic and somatotropic function, which can collectively inhibit further fat loss at the expense of lean tissues such as muscle and bone, as well as promoting visceral fat regain, thereby possibly increasing the risk of heart disease, diabetes, sarcopenia and osteoporosis.

In light of urgent worldwide efforts to find effective ways to maximize loss of excess fat (particularly visceral fat) while minimizing loss of lean tissues such as muscle and bone, as well as mounting interest in the use of very low energy diets and long-term energy restriction to prolong health and lifespan in humans **(5)**, future weight loss and energy restriction trials would benefit from knowledge of the effects of various interventions on body composition using sensitive measures, as well as key hormonal regulators of body composition.

Conflict of Interest Statement

Amanda Sainsbury-Salis is the author of *The Don't Go Hungry Diet* (Bantam, Australia and New Zealand, 2007) and *Don't Go Hungry For Life* (Bantam, Australia and New Zealand, 2011). None of the authors had a conflict of interest.

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