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Review

Role of the arcuate nucleus of the hypothalamus in regulation of body weight during energy deficit

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ABSTRACT

Acute or long-term energy deficit in lean or obese rodents or humans stimulates food intake or appetite and reduces metabolic rate or energy expenditure. These changes contribute to weight regain in post-obese animals and humans. Some studies show that the reduction in metabolic rate with energy deficit in overweight people is transient. Energy restriction has been shown in some but not all studies to reduce physical activity, and this may represent an additional energy-conserving adaptation. Energy restriction up-regulates expression of the orexigenic neuropeptide Y, agouti related peptide and opioids and down-regulates that of the anorexigenic alpha-melanocyte stimulating hormone or its precursor pro-opioomelanocortin and the co-expressed cocaine and amphetamine-regulated transcript in the arcuate nucleus of the hypothalamus. Recapitulating these hypothalamic changes in sated animals mimics the effects of energy deficit, namely increased food intake, reduced physical activity and reduced metabolic rate, suggesting that these energy-conserving adaptations are at least partially mediated by the hypothalamus.

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1. Introduction

Living organisms, from worms and flies to monkeys and human beings, show adaptive mechanisms in response to changes in food intake, body weight or adiposity that help to attenuate excessive deviations from optimum levels. This physiological drive to defend a 'set point' in body weight and adiposity helps to explain why obesity is so resistant to non-surgical treatments and – on the other hand – why many people gain weight slowly, if at all, despite large day to day variations in food intake and voluntary energy expenditure. However, whereas the current worldwide obesity epidemic would suggest that the adaptive mechanisms that prevent excessive weight gain during positive energy balance are easily overridden by inactivity and over consumption of readily accessible foods and beverages, the adaptive mechanisms that prevent weight gain below a certain threshold appear to be considerably more powerful. This is likely related to the fact that starvation is a more immediate threat to species survival than over nutrition. Indeed, whereas excessive weight loss or weight gain *both* threaten reproductive fitness and individual lives, starvation reduces fertility and kills in a matter of weeks to months whereas over consumption generally only hinders fertility once body mass index reaches the obese range, and it takes decades to die from the metabolic complications of obesity.

Multiple systems and mechanisms are involved in the critical process of preventing negative energy balance. For example, upon perception of energy deficit, adaptive changes in gut functions, in brain centers that regulate food hedonics and eating behavior, and in hypothalamic centers can collectively promote the restoration of energy balance. The pathways by which energy deficit is perceived by the organism involve signals from nutrients and hormones that converge and act directly on brain centers such as the hypothalamus, as recently reviewed (Sandoval et al., 2008; Ahima and Lazar, 2008). Adaptive changes in gut function in response to negative energy balance include increased circulating concentrations of the 'hunger hormone' ghrelin (Olszanecka-Glinianowicz et al., 2008), and decreased circulating concentrations of the 'satiety hormone' peptide YY (PYY) (Pfluger et al., 2007), and these changes could contribute to increased appetite. Additionally, negative energy balance has been reported to enhance food palatability (Cameron et al., 2008) and food hoarding behavior (Shizgal et al., 2001), demonstrating the diversity and complexity of adaptive responses that defend against energy deficiency. For the purposes of this review, however, we will concentrate on the adaptive mechanisms that attenuate weight loss during energy restriction in animals and humans, focusing on energy intake, energy expenditure due to basal metabolic rate and physical activity, and the role of the hypothalamus – particularly the arcuate nucleus (ARC) – in mediating these processes.

In brief, energy restriction in animals and humans is associated with increases in food intake or appetite and reductions in resting or total daily energy expenditure beyond those that would be expected from the associated losses in body mass. These changes have been shown to contribute to weight regain. Additionally, some studies suggest that energy restriction reduces physical activity levels, and this may also contribute to weight regain given that physical activity is a major determinant in successful weight maintenance in weight-reduced individuals.

Work in animals has shown that energy restriction leads to changes in expression of a variety of neuropeptides within the hypothalamus, and these changes likely contribute to the associated changes in food intake or appetite, energy expenditure and physical activity. Intriguingly, all of these energy-conserving adaptations have been shown to occur even in overweight and obese people after loss of as little as 6–12% of body weight (Doucet et al., 2000; Westerterp-Plantenga et al., 2001; Martin et al., 2007; Leibel et al., 1995; Hukshorn et al., 2003a) and may therefore contribute to the exceedingly low success rate of non-surgical treatments for overweight and obesity. Understanding the hypothalamic mechanisms for the energy-conserving adaptations that occur in response to energy deficit is likely to lead to more effective treatments for overweight and obesity.

2. Effects of energy deficit on hypothalamic regulators of energy balance

By knowledge of the hypothalamic changes occurring with fasting or energy restriction in animals, and knowledge of how these hypothalamic changes influence energy homeostasis, substantial insights have been gained into the mechanisms by which the hypothalamus regulates body weight. There are dozens of neuropeptides whose hypothalamic expression has been shown to be altered by experimentally induced 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 corticotropin-releasing hormone family of peptides and neurotensin (Kalra et al., 1999). For the purposes of this review we will focus on the following factors within the ARC and their role in the regulation of food intake, energy expenditure and physical activity: the orexigenic agents neuropeptide Y (NPY), agouti related peptide (AgRP) and the opioid peptides dynorphins, as well as the anorexigenic agent proopiomelanocortin (POMC), the precursor of alpha-melanocyte stimulating hormone (α-MSH) and the co-expressed cocaine and amphetamine-regulated transcript (CART), as summarized in Fig. 1.

2.1. Insights from fasting in animals

One line of evidence that a particular hypothalamic peptide plays a role in the regulation of energy homeostasis is the observation that energy restriction alters the hypothalamic expression of that peptide. The orexigenic NPY which acts via Y receptors and AgRP, which also induces orexigenic effects by antagonism of hypothalamic melanocortin (MC) receptors (Robinson et al., 2000), are co-expressed in neurons in the ARC (Chronwall et al., 1985; Broberger et al., 1998; Hahn et al., 1998) as shown in Fig. 1. These NPY-AgRP neurons in the ARC give rise to projections towards the paraventricular nucleus (PVN) as well as the dorsomedial nucleus (DMN) of the hypothalamus and the median preoptic area (Bai et al., 1985; Kerkerian and Pelletier, 1986). During food deprivation in rodents, unanimous research shows that the hypothalamic peptide content and mRNA expression levels of both NPY (Ziotopoulou et al., 2000; Swart et al., 2002; Brady et al., 1990; Kalra et al., 1991; Savontaus et al., 2002) and AgRP (Ziotopoulou et al., 2000; Swart et al., 2002; Savontaus et al., 2002; Mizuno and Mobbs, 1999) are increased. In addition to up-regulation of hypothalamic expression of the orexigenic NPY and AgRP, energy restriction in rodents up-regulates the expression of pre-prodynorphin, an orexigenic member of the opioid peptide family (Berman et al., 1997; Herve and Fellmann, 1997). As will be discussed below, these changes in hypothalamic expression of orexigenic agents can contribute to the appetite-promoting and energy-conserving effects of negative energy balance.

Whereas energy deficit up-regulates the hypothalamic expression of orexigenic peptides, it down-regulates that of anorexigenic agents. POMC in the ARC and the co-expressed CART are two such examples, and these are illustrated in Fig. 1. POMC is the precursor of α-MSH, which decreases feeding by agonizing central MC4 receptors (Benoit et al., 2000; McMinn et al., 2000;

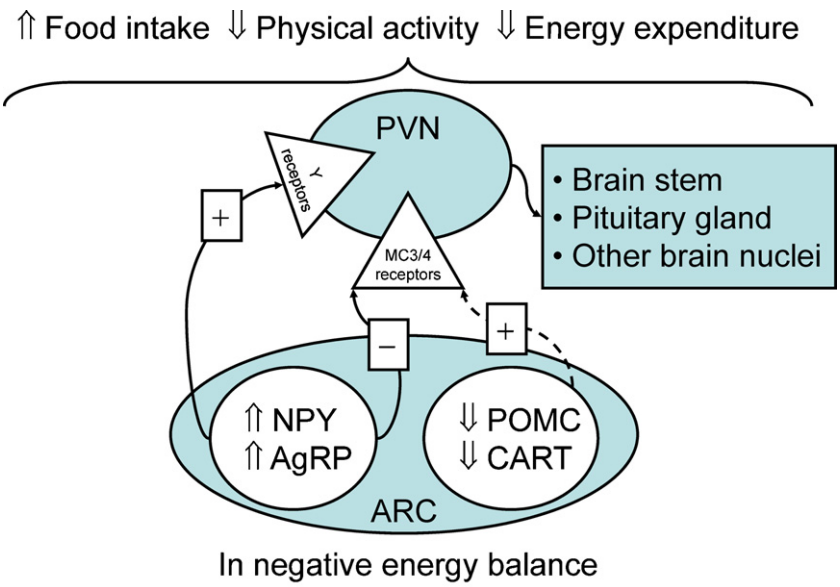

Fig. 1. The role of NPY/AgRP and POMC/CART in the ARC in the regulation of energy homeostasis. Under conditions of negative energy balance, expression of NPY and AgRP in the ARC are increased while that of POMC and CART are reduced. Subsequently, enhanced action of NPY on Y receptors such as Y1 and Y5 in other hypothalamic nuclei, notably the PVN, induces effects on food intake, physical activity and energy expenditure via multiple mechanisms involving the brain stem, the pituitary gland (e.g. the hypothalamo-pituitary thyroid axis) and other brain nuclei. Additionally, enhanced AgRP antagonism of MC receptors, notably MC4 receptors, and reduced agonism of these receptors by α-MSH, a product of the POMC gene, promotes orexigenic and energy-conserving effects via complex neuronal relays.

Vrang et al., 1999). CART has been shown to have anorexigenic as well as orexigenic effects. Hypothalamic expression levels of both POMC and CART are decreased during food deprivation in rodents (Ziotopoulou et al., 2000; Swart et al., 2002; Brady et al., 1990; Savontaus et al., 2002; Mizuno et al., 1999; Kristensen et al., 1998; Ahima et al., 1999).

In summary, food deprivation alters the expression levels of hypothalamic regulators of energy balance with an up-regulation of orexigenic neuropeptides and a down-regulation of anorexigenic neuropeptides. These changes may be critical in mediating energy-conserving mechanisms and enhancing survival during energy deficit.

2.2. Insights from genetically obese rodents

In addition to short-term fasting studies in rodents, another line of evidence that a particular hypothalamic peptide plays an important role in the regulation of energy homeostasis is observation that the hypothalamic expression of that peptide is altered in obese rodent models. For this purpose, rodents that are deficient in leptin or the action of leptin have been used extensively as a model of chronic energy deficit with concurrent obesity.

Leptin is the hormone product of the mouse ob gene and human homologue gene, LEP (Zhang et al., 1994). Leptin is secreted predominantly by adipocytes and its expression is positively influenced by the amount of energy stored in fat (Maffei et al., 1995; Frederich et al., 1995; Considine et al., 1996; Hamilton et al., 1995; Lönnqvist et al., 1995). The initial view of leptin as an anti-obesity hormone (Friedman and Halaas, 1998; Flier, 1998; Spiegelman and Flier, 1996) based on the observation that administration of leptin to rodents decreases food intake, increases energy expenditure and enhances lipid oxidation (Halaas et al., 1995; Pelleymounter et al., 1995; Campfield et al., 1995; Shimabukuro et al., 1997) has been challenged due to the inability of high levels of endogenous or exogenous leptin to induce the same effects in obese patients and other obese mammals (Maffei et al., 1995; Frederich et al., 1995; Considine et al., 1996; Van Heek et al., 1997). On the other hand, there is a significant decrease in circulating leptin levels during energy deficit (Frederich et al., 1995; Ahima et al., 1996). This fall in leptin with fasting has been demonstrated to be an important mediator of the neuroendocrine adaptations to energy restriction (Legradi et al., 1997, 1998; Yu et al., 1997; Finn et al., 1998; Bray and York, 1979). Together with the leptin resistance of obesity, it seems that the dominant role of leptin in energy homeostasis is as a mediator of the adaptations to energy deficit (Ahima et al., 1996; Bray and York, 1979; Ahima and Flier, 2000; Jequier, 2002). Indeed, rodent models with leptin deficiency such as *ob/ob* mice, or leptin receptor deficiency such as *db/db* mice or *fa/fa* (Zucker) rats, have similar behavioral, metabolic and neuroendocrine abnormalities as those resulting from energy deficit such as hyperphagia, hypometabolism and a variety of neuroendocrine defects including infertility and profound hypercorticosteronemia and are associated with early onset morbid obesity and diabetes (Halaas et al., 1995; Campfield et al., 1995; Ahima et al., 1996; Bray and York, 1979). Thus leptin-deficient and leptin receptor-deficient rodents are a model of chronic energy deficit with concomitant obesity.

Hypothalamic NPY gene expression and secretion is upregulated in *ob/ob* and *db/db* mice and *fa/fa* rats (Wilding et al., 1993; Schwartz et al., 1996; Beck, 2000; Sanacora et al., 1990; Kowalski et al., 1999). In addition to changes in NPY expression and secretion, functionality of the NPY-ergic system is modified in rodents with deficient leptin signaling. Thus the insulin-induced reduction in hypothalamic NPY mRNA expression seen in lean rats is abolished in *fa/fa* rats (Schwartz et al., 1991). Moreover, NPY is released in an exacerbated and anarchic manner in *fa/fa* rats (Stricker-Krongrad et al., 1997; Dryden et al., 1995) rather than showing a peak release during the transition from the light to the dark phase as in lean counterparts (Jhanwar-Uniyal et al., 1990). This may contribute to the disappearance of the rhythm of feeding behavior between dark and light phases in *fa/fa* rats (Beck, 2000; Becker and Grinker, 1977). Most significantly, the importance of NPY in mediating the syndrome associated with leptin deficiency is seen by attenuation of the hyperphagia and the neuroendocreine defects and obesity of *ob/ob* mice by NPY knockout (Erickson et al., 1996a; Marsh et al., 1998).

In addition to up-regulation of the hypothalamic NPY-ergic system in mice deficient in leptin signaling, hypothalamic expression of the orexigenic AgRP is increased five to ten fold in *ob/ob* and *db/db* mice (Mizuno and Mobbs, 1999; Shutter et al., 1997). Additional orexigenic peptides whose hypothalamic expression is up-regulated by leptin deficiency in *ob/ob* mice are endogenous opioids. The endogenous opioid system is composed of a family of three peptides; endorphins, enkephalins, and dynorphins, which act predominantly on mu (μ), delta (δ) and kappa (κ) opioid receptors, respectively (Glass et al., 2000). Thus *ob/ob* mice have a 5-fold increase in dynorphin peptide level in the DMN and a 2–5-fold increase in β-endorphins in the DMN, PVN as well as the ventromedial nucleus of the hypothalamus (VMN) (Khawaja et al., 1991). Additionally, *ob/ob* mice show a significant increase in sensitivity to κ-opioid receptor-preferring ligands with respect to glucoregulatory functions (Khawaja et al., 1990).

Whereas the hypothalamic expression, secretion or function of orexigenic peptides is up-regulated by leptin deficiency in rodents, anorexigenic hypothalamic peptides are down-regulated in rodents with leptin deficiency. Thus hypothalamic POMC and CART mRNA levels are 50% and 15% lower in *ob/ob* mice than in lean counterparts respectively (Thornton et al., 1997; Schwartz et al., 1997; Mizuno et al., 1998; Duan et al., 2007).

These data demonstrate that in rodent models of concomitant energy deficit and obesity due to deficiency in leptin signaling, the hypothalamus shows increased expression or secretion of orexigenic neuropeptides and decreased expression of anorexigenic neuropeptides.

2.3. Insights from humans

While it is not currently feasible to investigate hypothalamic expression or secretion of NPY, AgRP, opioids, POMC/α-MSH or CART in humans during dynamic changes in energy balance, existing data suggests that – as in animals – these hypothalamic peptides are involved in the regulation of energy homeostasis in humans. For instance, people with anorexia nervosa who are underweight and malnourished have been found to have significantly increased concentrations of NPY in the cerebrospinal fluid compared to normal weight controls, and this change is significantly correlated with the decrease in serum and cerebrospinal fluid leptin concentrations (Kaye et al., 1990; Martinez et al., 1993; Hebebrand et al., 1995; Grinspoon et al., 1996; Baranowska et al., 1997; Ferron et al., 1997; Mantzoros et al., 1997). Moreover, the elevated cerebrospinal fluid NPY levels in people with anorexia nervosa are normalized when their eating behavior and body weight are restored to normal, suggesting that increases in central NPY levels may be a biological adaptation to energy deficit in humans (Kaye et al., 1990; Gendall et al., 1999) as well as in rodents. In keeping with this, a polymorphism in human preproNPY, a p.L7P substitution, has been shown to cause altered NPY secretion, and association studies suggest that this functional substitution may be a strong independent risk factor for various metabolic and cardiovascular diseases (Pesonen, 2008).

Further evidence that the hypothalamic factors implicated in energy homeostasis in rodents are also important in humans comes from the fact that mutations in these genes in humans are linked to obesity. For instance, people who have compound heterozygous mutations in the POMC gene or the gene for the enzyme that converts POMC to α-MSH (proconvertase or PC1) are clearly obese (Clement, 2000). In addition, several dominant mutations in the MC4 receptor gene cause early onset obesity, and it is estimated that MC4 receptor mutations may be a relatively common cause of excessive adiposity amongst people who are severely obese (Clement, 2000). Additionally, a CART missense mutation has been shown to co-segregate with severe obesity through three generations in a human family, and polymorphisms in the CART gene may influence fat distribution and are associated with obesity in humans (Murphy, 2005).

Taken together, these findings illustrate the important contribution of NPY, the melanocortin system and CART to energy homeostasis in humans.

3. Effects of energy deficit and the resultant hypothalamic changes on appetite

Energy deficit, or perceived energy deficit as in rodents lacking leptin signaling, are associated with changes in hypothalamic peptide expression as discussed in Section 2. One of the major pathways through which these hypothalamic changes promote positive energy balance is by stimulating food intake. Unanimous research shows that energy deficit, or the hypothalamic changes elicited by energy deficit, lead to robust increases in food intake or appetite in rodents and humans and that this change promotes weight regain.

3.1. Effects of energy deficit on food intake in rodents

Rodent studies have clearly shown that negative energy balance results in compensatory increases in food intake that promote weight regain. In lean mice, 48 h of food deprivation results in an 18% reduction in body weight and a 65% increase in daily food intake compared to that measured prior to fasting, and this fasting-induced hyperphagia persists for up to 5 days (Erickson et al., 1996b). Not only is hyperphagia seen in lean animals after acute fasting, it also occurs in obese animals after less severe but longer-term energy restriction. In obesity-prone rats that were rendered obese by *ad libitum* access to a high fat diet, reducing energy intake to 43% of *ad libitum* intake for 2 weeks resulted in a 15% loss of body weight (MacLean et al., 2004). However, when these rats were allowed *ad libitum* access to a low fat diet for 8 weeks, energy intake increased to levels measured on the high fat diet prior to weight reduction, and body weight returned towards the growth curve of age-matched obesity-prone rats that had continuous *ad libitum* access to the high fat diet (MacLean et al., 2004). Although weight reduction in these obese rats reduced energy expenditure and as such would tend to promote weight regain as described in Section 5.1, the major factor driving their weigh regain was increased energy intake, because weight-reduced obese rats that followed an energy restricted maintenance diet for 8 weeks maintained their lower weight (MacLean et al., 2004).Taken together, these data demonstrate the pivotal role of hyperphagia in restoring usual body weight after energy restriction in lean and obese rodents.

3.2. Effects of energy deficit on appetite in humans

As might be expected, severe energy restriction in lean people results in compensatory increases in food intake. In the Minnesota Experiment by Ancel Keys and colleagues, 24 weeks of semi-starvation resulting in a 25% reduction in body weight in lean young men lead to a 160% increase in energy intake relative to pre-starvation intake (Keys et al., 1950). Similar findings have been shown after short-term energy restriction. In the two days after 48 h on a restricted diet consisting of 38% of their estimated maintenance energy requirements and resulting in a 1.1 kg weight loss, lean young men ate 143% and 124% of their estimated energy requirements each day, respectively (Mars et al., 2005). Even in overweight people, losing weight by severely restricting energy intake increases measures of appetite such as hunger, desire to eat and estimates of prospective consumption (Mars et al., 2005; Hukshorn et al., 2003b). While it is often assumed that hunger is only a problem associated with very low energy diets or 'crash dieting', or with energy restriction in lean individuals, significant increases in appetite or hunger have also been measured in obese

people losing weight by mild energy restriction, or by mild energy restriction combined with physical activity (Doucet et al., 2000; Westerterp-Plantenga et al., 2001). This increased appetite probably contributes to the high rate of attrition of weight loss attempts, since the degree of hunger experienced by overweight people after an energy restricted diet is a significant predictor of subsequent weight regain (Pasman et al., 1999).

3.3. Effects of hypothalamic regulators of energy balance on food intake

The effects of energy restriction on food intake or appetite can be recapitulated in sated animals by experimentally mimicking the associated hypothalamic changes. This therefore suggests that it is these hypothalamic changes that contribute to the hyperphagia or increased appetite in response to restricted food intake. For instance, the orexigenic agents NPY (Stanley et al., 1986), AgRP or synthetic MC4 receptor antagonists (Hagan et al., 2000; Kim et al., 2000a; Kask et al., 1999) increase body weight and/or fat mass and feeding. In addition to these orexigenic effects of NPY and AgRP, endogenous opioids may also contribute to the energy-conserving, appetite-promoting effects of negative energy balance. Administration of opioid receptor agonists to animals (and humans) results in robust and sustained increases in food intake and body weight, whereas opioid receptor antagonists have the opposite effect (Glass et al., 2000). The dynorphins and their κ-opioid receptors are strongly implicated in the effects of opioids on feeding and body weight. Indeed, selective blockade of κ-opioid receptors significantly reduces fasting-induced hyperphagia in rats (Lambert et al., 1993), and reduces food intake and body weight in obese rodents models (Cole et al., 1995; Jarosz and Metzger, 2002).

In comparison to the hyperphagic effects of NPY, AgRP and opioids such as dynorphins, hypothalamic administration of the anorexic peptides α-MSH or analogs (Thiele et al., 1998; Brown et al., 1998) or CART-related peptides (Kristensen et al., 1998; Murphy, 2005; Larsen et al., 2000) to rodents reduce body weight and/or fat mass in association with inhibition of food intake. Interestingly, the studies in which CART was shown to have an anorexigenic or weight-reducing effect in fed or 24-h fasted rodents used the intracerebroventricular route of administration (Murphy, 2005), but this was shown to induce behavioral defects that could interfere with food intake (Abbott et al., 2001). Contrarily, recent studies have shown that administering minute doses of CART directly into specific hypothalamic nuclei such as the ARC and PVN of 24-h fasted rats increases food intake without inducing the behavioral abnormalities seen after intracerebroventricular administration (Abbott et al., 2001). Moreover, long-term overexpression of CART in the PVN of normal rats using an adeno-associated virus expressing CART increased food intake and body weight, particularly under high fat-fed conditions (Smith et al., 2008). It is therefore likely that CART has dual effects on food intake and energy balance depending on the brain region in which it acts and the behavioral alterations it may induce.

The effects of these agents on body weight and/or adiposity are not simply due to the associated changes in food intake, but can also be ascribed to changes in energy efficiency. Indeed, when the food intake of animals that receive hypothalamic administration of these agents is matched in control animals (i.e. the food intake of infused animals is restricted to that consumed by controls, or the food intake of control rats is restricted to the intake of infused animals), the effects to promote changes in energy balance or adiposity persist (Zarjevski et al., 1993; Small et al., 2001; Cettour-Rose and Rohner-Jeanrenaud, 2002; Rohner-Jeanrenaud, 1995). Clearly, therefore, changes in food intake alone do not fully account for the associated changes in body weight or fat mass, and hypothalamic peptides have additional effects besides alterations in food intake.

4. Effects of energy deficit and the resultant hypothalamic changes on physical activity

Although increased food intake is an important mediator of weight gain after periods of energy deficit or during hypothalamic manipulations that mimic energy deficiency, it is not the only mechanism promoting positive energy balance. Studies in which hypothalamic manipulations that mimic energy deficiency result in decreased physical activity suggest that reduced physical activity could contribute to the tendency to conserve energy during periods of negative energy balance. Some studies show that energy deficiency reduces physical activity in animals and humans, but this has not been extensively studied nor is it a universally observed phenomenon.

4.1. Effects of energy deficit on physical activity in rodents

Studies on the effects of 48–72-h fasting on physical activity in rodents have given conflicting results, indicating increased (Overton et al., 2001), decreased (Williams et al., 2000) or no change in physical activity (Nagashima et al., 2003). During long-term chronic food restriction in rodents however, an increase in physical activity is generally observed. Using an electronic device attached to the home cage, increased physical activity was reported in aged and chronically energy restricted male and female rats compared to age- and gender-matched *ad libitum* fed controls (Duffy et al., 1989, 1990a). Similar results were obtained in mice (Duffy et al., 1990b). Longitudinal analysis of home cage activity showed that the age-related decline in physical activity in *ad libitum* fed rats was prevented by chronic energy restriction (Yu et al., 1985). Moreover, when access to a running wheel was given, chronically food-restricted rats and mice showed higher levels of wheel running activity than *ad libitum* fed control animals (Goodrick et al., 1983a; Ingram et al., 1987). In fact, under certain experimental conditions, rats that are given access to food for a limited period of time each day as well as free access to a running wheel actually run so much that they die of starvation (Gutierrez et al., 2002). Interestingly, intermittent food restriction differentially affected wheel running activity across the life span in male rats (Goodrick et al., 1983b). Thus wheel running activity was lower in the energy restricted group early in life but was higher in the same group later in life compared to *ad libitum* fed age-matched controls (Goodrick et al., 1983b), suggesting that the influence of energy deficit on physical activity may change with ageing. A similar outcome was seen in young and old non-human primates as will be discussed below. Age differences may explain some of the discrepancies in results between studies showing no change or a decrease in physical activity compared to *ad libitum* fed controls (Evans et al., 2005; Dulloo and Girardier, 1993). Taken together, the most consistent finding in rodents is that physical activity increases during long-term energy deficit. It is not yet clear why this is so, but in some paradigms increased physical activity in the face of energy restriction may be related to increased food-seeking behavior; in other paradigms it may be related to maintenance of body temperature in the face of the energy deficit-induced reduction in body temperature (Gutierrez et al., 2002).

4.2. Effects of energy deficit on physical activity in humans and non-human primates

Reports on the effects of energy deficit on physical activity in humans are sparse and there are difficulties in accurately assessing physical activity in free-living conditions. Moreover, spontaneous

physical activity levels measured in metabolic chambers have little correlation with free-living physical activity levels as indicated by the ratio of total energy expenditure measured by double-labeled water versus basal metabolic rate (Martin et al., 2007). Using this technique, 25% energy deficit in overweight men and women induced by food restriction for 3 months was shown to reduce free-living physical activity levels (Martin et al., 2007). In another study, however, overweight subjects on a diet-only intervention to achieve weight loss of over 10 kg and a body mass index of under 25 kg/m^2 showed reduced physiological stress in response to exercise and a tendency to be more physically active than they had been prior to the intervention (Weinsier et al., 2000a). It is important to note, however, that in the study reporting reduced free-living physical activity in response to energy deficit, subjects were studied while in energy deficit (Martin et al., 2007). Contrarily, in the study reporting improvements in exercise response and activity levels, subjects were studied after a period of 4 weeks in energy balance (Weinsier et al., 2000a). Given that certain energy-conserving adaptations to energy deficit, notably reduced metabolic rate and thyroid function, are seen during energy restriction but not after a period of 10 days to 3 months in energy balance in weight-reduced individuals (Weinsier et al., 2000b; Westerterp-Plantenga et al., 2004), this difference in testing schedule probably contributes to the discrepancy in findings, in addition to other differences such as the method used to determine free-living physical activity. In order to determine whether energy deficit *per se* has effects on physical activity in humans independently of the effect of weight reduction to improve mobility, it is necessary to investigate subjects while they are still in a state of negative energy balance.

There are a few studies that have investigated the effects of long-term energy restriction on physical activity in non-human primates. The results are variable. Adult rhesus moneys (9–10 years of age) submitted to 70% energy restriction for 1 year showed significant reductions in body weight, body fat and physical activity levels compared to *ad libitum* fed controls (Kemnitz et al., 1993). When energy restriction in adult monkeys was prolonged for 5–6 years, however, no difference in physical activity (Ramsey et al., 1997), or a trend to increased physical activity (Moscrip et al., 2000), was reported. Similarly, a comparable (Moscrip et al., 2000) or increased (DeLany et al., 1999) level of physical activity was reported in old monkeys (17–22 years of age) who had been on an energy restricted diet for 5–10 years compared to *ad libitum* fed controls. Given that adaptive energy-conserving responses to energy deficit can be temporary as discussed above (Weinsier et al., 2000b; Westerterp-Plantenga et al., 2004), differences in the timing of measurements in these non-human primate studies may account for these discrepancies. Nevertheless, energy restriction in young monkeys (i.e. 2–2.5 years of age) for 5–6 years consistently reduced physical activity (Weed et al., 1997; Moscrip et al., 2000), similar to the results seen in young versus old rodents (Goodrick et al., 1983a).

Taken together, a limited number of studies have shown that energy restriction is associated with reduced physical activity in young rodents and in young non-human primates, as well as in one study in overweight men and women in which physical activity was measured during energy restriction. This may represent an energy-conserving adaptation to reduced energy availability, in keeping with the effects of hypothalamic regulators of energy homeostasis such as NPY, MC4 receptor action and CART on physical activity as discussed below. Contrarily, other studies have shown that energy restriction or weight loss in rodents, non-human primates and humans is associated with no change or an increase in physical activity. This may represent increased activity due to food-seeking behavior, maintenance of thermoregulation or increased mobility due to weight loss. Given that any effects of energy deficit on physical activity, if present, may be transient (Weinsier et al., 2000b; Westerterp-Plantenga et al., 2004), physical activity needs to be measured *during* energy deficit in order to determine whether there is any effect of energy deficit *per se* on physical activity levels.

4.3. Effects of hypothalamic regulators of energy balance on physical activity

Reductions in physical activity in response to energy deficit, where present, could be mediated by the effects of energy deficit on hypothalamic peptides. It is well established that spontaneously genetically obese rodents, which demonstrate increased hypothalamic expression of NPY and AgRP (Shutter et al., 1997; Makimura et al., 2000) concomitant with decreased expression of POMC and CART (Kristensen et al., 1998; Makimura et al., 2000) exhibit reduced physical activity compared to lean control animals (Butler and Cone, 2001). Moreover, intracerebroventricular administration of NPY (Heilig et al., 1989), or MC4 receptor knockout in rodents (Ste Marie et al., 2000) have been shown to reduce locomotion. In contrast, CART (Kimmel et al., 2000) has been shown to increase locomotor activity after infusion into the cerebral ventricles of rats, but these findings must be interpreted in light of the finding that intracerebroventricular but not intrahypothalamic administration of CART induces behavioral abnormalities that can impact on food intake and physical activity (Abbott et al., 2001).

5. Effects of energy deficit and the resultant hypothalamic changes on energy expenditure

Longer-term non-surgical clinical weight loss trials typically result in weight losses of 5–14 kg/year, and most of that weight loss is achieved within the first months despite efforts to ensure volunteers' compliance with physical activity and/or dietary prescriptions. While lack of compliance with lifestyle prescriptions is commonly recognized in clinical weight loss trials (perhaps related to the effects of energy deficit to increase appetite and reduce physical activity), the universality of the weight loss 'plateau' even in tightly controlled clinical research settings suggests metabolic factors preventing further weight loss. Indeed, studies from rodents and humans show that energy restriction and weight loss lead to marked and significant decreases in metabolic rate and energy expenditure. This energy-conserving adaptation is a significant predictor of subsequent weight regain in weight-reduced rodents (MacLean et al., 2004; Dulloo and Girardier, 1990) and people (Pasman et al., 1999; Goran, 2000). Rodent studies suggest that this adaptation is mediated by the hypothalamic changes that occur in response to energy deficit.

5.1. Effects of energy deficit on energy expenditure in rodents

In animals, energy expenditure has been estimated by parameters such as oxygen consumption, heat production, body temperature or expression of uncoupling proteins in muscle or fat. An acute 24-h fast that resulted in an approximately 25% reduction in body weight in mice reduced resting metabolic rate by 49% compared to that measured in mice allowed to eat *ad libitum* (Bezaire et al., 2001). Similarly in rats, a 48–72-h period of food deprivation induced a 10–15% reduction in body weight and a significant reduction in 24-h oxygen consumption and light-phase core body temperature compared to that measured prior to fasting (Williams et al., 2000; Nagashima et al., 2003; Bezaire et al., 2001). Importantly, this reduction in oxygen consumption during food deprivation is largely independent of concurrent changes in locomotor activity, suggesting a reduction in resting metabolic rate due to negative energy balance *per se* (Nagashima et al., 2003). In addition to marked effects of acute energy restriction in rodents, reduced energy expenditure also occurs during longer-term energy restriction. In lean rats, chronic energy restriction for

several months that resulted in a 40% decrease in body fat mass gave rise to an 8% decrease in daily energy expenditure that could not be accounted for by changes in metabolic tissue mass or physical activity (Dulloo and Girardier, 1993). In obesity-prone rats, where marked obesity was induced by 16 weeks on a high fat diet, 60% energy restriction for 2 weeks resulting in a 14% weight loss was accompanied by significantly suppressed energy expenditure (MacLean et al., 2004, 2006). This decrease in energy expenditure in energy restricted rats remained significant compared to that in rats with continuous *ad libitum* access to food even after adjustment for changes in body composition (MacLean et al., 2004, 2006), suggesting an increased metabolic efficiency/decreased metabolic rate during negative energy balance. Interestingly, the reduced metabolic rate observed in these dietary-induced obese rats persisted throughout a period of weight maintenance for 8 weeks, thus restricted energy intake was required to achieve weight maintenance (MacLean et al., 2004; Dulloo and Calokatisa, 1991). This elevated energy efficiency contributed to rapid weight regain in these obesity-prone animals when *ad libitum* access to food was resumed (MacLean et al., 2004; Dulloo and Girardier, 1990). Take together, these studies demonstrate that acute or longer-term energy deficit lead to adaptive decreases in energy expenditure or metabolic rate in lean or obesity-prone rodents that are beyond the changes that would be expected from changes in locomotion or body mass. This metabolic adaptation predisposes towards weight restoration after energy restriction in rodents.

5.2. Effects of energy deficit on energy expenditure in humans

In accordance with studies in rodents, severe energy restriction in lean people results in compensatory reductions in metabolic rate. In the Minnesota Experiment by Ancel Keys et al, 24 weeks of semi-starvation resulting in a 25% reduction in body weight in lean young men lead to 25% decrease in metabolic rate relative to pre-starvation intake (Keys et al., 1950). Reductions in energy expenditure or metabolic rate have also been observed in overweight or obese people after weight loss and when measured during negative energy balance. For instance, overweight women showed a drop in absolute resting metabolic rate of 840 kJ/day (200 kcal/day) after having reduced their weight to normal (Weinsier et al., 2000b). Although part of the observed decrease in metabolic rate was due to the lower energy cost of a smaller body size, the reduction was significant even after adjusting for fat free mass and fat mass (Weinsier et al., 2000b). Similarly, severely obese men and women showed 10–30% decreases in resting energy expenditure, total 24-h energy expenditure and non-resting energy expenditure (adjusted for fat free mass) after losing 10% of their body weight by a very low energy diet (Rosenbaum et al., 1997). After losing an additional 10% of their body weight, these decreases were even more pronounced (Rosenbaum et al., 1997) and accounted for a drop in total energy expenditure of 3700 kJ/day (890 kcal/day) (Leibel et al., 1995). Similar reductions in resting metabolic rate or total energy expenditure have been observed in other studies in overweight or obese people after weight loss (Westerterp-Plantenga et al., 2001; Martin et al., 2007; Hukshorn et al., 2003b; Westerterp-Plantenga et al., 2004; Menozzi et al., 2000). This weight loss-induced drop in energy expenditure – like the associated increase in appetite – is a significant predictor of subsequent weight regain (Pasman et al., 1999; Goran, 2000).

It's important to note that not all clinical studies that have investigated energy expenditure in people after weight reduction have shown significant reductions in metabolic rate or energy expenditure beyond that which would be expected from the associated reduction in body mass. For instance, an average weight loss of 12.8 kg in black and white women, achieved through diet without exercise, was associated with reductions in sleeping and resting energy expenditures that were entirely attributable to changes in body composition (Weinsier et al., 2000a). Additionally, when post-obese men and women were studied after loss of 23–139 kg, 24-h energy expenditure and sleeping metabolic rate – when adjusted for body mass – were at least as high as in matched controls who had never been obese (Larson et al., 1995). In both of these studies, however, subjects were studied at 4 weeks (Weinsier et al., 2000a) or between 2 months to 6 years (Larson et al., 1995) after weight loss. Moreover, it has been shown that whereas weight loss in overweight men or women results in reductions in resting metabolic rate adjusted for body composition when these parameters are measured while subjects are still in negative energy balance, this difference is no longer observed when subjects are studied again after 10 days (Weinsier et al., 2000b) or 3 months in energy balance (Westerterp-Plantenga et al., 2004). This 'reversal' phenomenon was not seen in obese men and women after a period of 2 weeks of weight maintenance after loss of 10% of their body weight (Rosenbaum et al., 2000; Weinsier et al., 2001). Nonetheless, conclusions as to whether or not particular study populations of overweight or obese individuals show adaptive changes in metabolic rate or energy expenditure in response to weight loss need to make clear distinctions between measurements made in people during negative energy balance versus measurements made in people who have been in weight maintenance for a period of time.

In brief, energy deficit, whether short-term or longer-term, in rodents or in humans, in lean or obese individuals, reliably reduces indices of metabolic rate or energy expenditure even after adjusting for changes in body composition. In those clinical studies where weight loss was not associated with reductions in metabolic rate or energy expenditure, the discrepancy is likely due to the fact that subjects were studied at several days to years after weight loss, whereas the reduction in metabolic rate with weight loss in humans has been shown in some but not all human studies to be transient.

5.3. Effects of hypothalamic regulators of energy balance on energy expenditure

A large body of rodent studies have shown that changes in the central expression of hypothalamic regulators of energy homeostasis result in changes in energy expenditure as indicated by alterations in oxygen consumption, heat production, body temperature or expression of uncoupling proteins in muscle or fat. Notably, the orexigenic peptides NPY or AgRP (or MC4 receptor antagonism) not only increase food intake but also reduce parameters related to energy expenditure. Intracerebroventricular or intrahypothalamic NPY administration decreases core temperature (Szreder et al., 1994; Currie and Coscina, 1995) and reduces oxygen consumption (Hwa et al., 1999). Central NPY administration decreases sympathetically mediated thermogenic activity in brown adipose tissue (Kotz et al., 1998; Egawa et al., 1991), most likely by decreasing the expression of uncoupling protein-1 (Kotz et al., 1998). MC4 receptor inactivation has similar effects to NPY on energy expenditure: MC4 receptor knockout mice exhibit significant reductions in oxygen consumption (Ste Marie et al., 2000), and intracerebroventricular administration of AgRP or MC4 receptor antagonists to rats decrease the mRNA and peptide levels of uncoupling protein 1 in brown adipose tissue (Small et al., 2001; Baran et al., 2002).

In contrast to the effects of the orexigenic peptides NPY and AgRP to inhibit energy expenditure, the anorexigenic agent α-MSH appears to increase energy expenditure. Central administration of the α-MSH analogue MTII increased body temperature (Murphy et al., 2000), oxygen consumption (Hamilton and Doods, 2002), as well as expression of uncoupling proteins in brown adipose tissue and muscle relative to control values (Cettour-Rose and Rohner-Jeanrenaud, 2002). The effects of CART on metabolic rate

are not yet clear. Most studies would suggest a decreased metabolic efficiency after intrahypothalamic or intracerebroventricular CART administration to normal or obese rats, indicated by increased expression of uncoupling proteins 1, 2, and 3 in brown and white adipose tissue or muscle (Wang et al., 2000), increased lipid oxidation (Rohner-Jeanrenaud et al., 2002), a higher thermogenic response to a β3 agonist, increased uncoupling protein-1 mRNA expression in brown adipose tissue and significantly greater weight loss in response to 24-h fasting (Kong et al., 2003). Another study, however, has suggested that intracerebroventricular CART administration reduces metabolic rate, as indicated by a decrease in oxygen consumption (Asakawa et al., 2001), but this effect may be related to the fact that CART, particularly in higher doses and when administered intracerebroventricular but not intrahypothalamically, induces motor disturbances (Larsen et al., 2000; Abbott et al., 2001) which could inhibit food intake, physical activity and metabolic rate.

One mechanism via which changes in expression of hypothalamic regulators of energy homeostasis may reduce energy expenditure during energy deficit is through reduced thyroid function. Indeed, during energy deficit thyroid function is inhibited at multiple levels of the hypothalamo-pituitary-thyroid axis in rodents (Ahima et al., 1996; Blake et al., 1992; Boelen et al., 2006; Gavin et al., 1980), in healthy men and women (Boelen et al., 2008), as well as in overweight or obese individuals (Hukshorn et al., 2003a; Weinsier et al., 2000b; Rosenbaum et al., 2000; Wadden et al., 1990; Naslund et al., 2000; Douyon and Schteingart, 2002). As thyroid function is a major positive regulator of energy expenditure, this change could contribute to reduce energy expenditure during energy deficit. Enhanced hypothalamic expression of the orexigenic NPY and AgRP with energy deficiency likely inhibits the thyroid axis, because central administration of NPY, AgRP or MC4 receptor antagonists to normal rodents significantly reduces function of the thyrotropic axis at several levels (Small et al., 2001; Fekete et al., 2001; Kim et al., 2000b). Neuronal endings containing NPY- and AgRP-immunoreactivity have been detected in close association with the thyrotropin releasing hormone-synthesizing neurons of the hypothalamic paraventricular nucleus in rodents (Legradi and Lechan, 1999, 1998) and in man (Mihaly et al., 2000), suggesting direct inhibitory effects of NPY and AgRP on the thyrotropic axis at the level of the hypothalamus.

In summary, up-regulation in hypothalamic expression of the orexigenic peptides NPY and AgRP with energy deficit or in leptin-deficient obesity syndromes in rodents could induce metabolic changes consistent with reduced energy expenditure. Conversely, the anorexigenic agent POMC/α-MSH and the co-expressed CART induce metabolic changes consistent with increased energy expenditure after intracerebroventricular or intrahypothalamic administration, so the reductions in hypothalamic expression of these agents with energy deficit or leptin deficiency would also be expected to promote a hypometabolic state. These hypothalamic effects on energy expenditure may be mediated via changes such as reductions in activity of the hypothalamo-pituitary-thyroid axis, and could contribute to the inefficiency in weight loss that has been reported during longer-term negative energy deficit – particularly more extreme energy deficit – in obese humans (Sweeney et al., 1993).

6. Summary and conclusions

Although obesity is associated with a significantly greater risk of diseases such as diabetes, and although weight loss is associated with significant reductions in disease risk factors, most people who lose excess weight by lifestyle means regain the weight they lost.

A possible explanation for the attrition in weight loss attempts is that the body – even in obesity – defends against negative energy balance with compensatory mechanisms that protect against further weight loss. Energy deficit – whether acute or long-term, in rodents or in humans, in lean or in obese individuals – stimulates food intake or appetite and reduces metabolic rate or energy expenditure. These changes have been shown to contribute to weight regain in lean or post-obese animals and humans. Some but not all studies have shown that the reduction in metabolic rate that occurs in response to energy deficit in overweight or obese humans is transient, and this must be taken into consideration when making conclusions as to whether or not a particular study group exhibits compensatory reductions in metabolic rate or energy expenditure in response to energy deficit or weight loss that would predispose to weight regain.

In addition to effects on energy intake and metabolic rate or energy expenditure, a limited number of studies have shown that energy restriction is associated with reduced physical activity in young rodents and in young non-human primates, as well as in one study in overweight men and women in which physical activity was measured during energy restriction. This may represent an energy-conserving adaptation to reduced energy availability. Contrarily, other studies have shown that energy restriction or weight loss in rodents, non-human primates and humans is associated with no change or an increase in physical activity. The reasons for this are not clear, but may involve increased activity due to food-seeking behavior, thermoregulation or increased mobility due to weight loss. Given that any effects of energy deficit on physical activity, if present, may be transient, physical activity needs to be measured *during* energy deficit in order to determine whether there is any effect of energy deficit *per se* on physical activity levels.

In the ARC, energy restriction up-regulates hypothalamic expression of the orexigenic NPY, AgRP and opioids such as dynorphins and down-regulates that of the anorexigenic α-MSH or its precursor POMC and the co-expressed CART. Experimentally recapitulating these hypothalamic changes in fed animals or in rodents deficient in leptin or leptin receptors has been shown to mimic the increased food intake, reduced physical activity and reduced metabolic rate that have been observed during energy deficit. Taken together, these data suggest that the energy-conserving adaptations to energy deficit are mediated – at least in part – by the hypothalamus.

Given that longer-term non-surgical clinical weight loss trials typically result in weight losses of 5–14 kg/year and that most of that weight loss is achieved within the first months despite efforts to ensure volunteers' compliance with physical activity and/or dietary prescriptions, and given the proven role of increased appetite and reduced metabolic in weight regain and the importance of physical activity for weight maintenance, targeting the hypothalamic pathways that stimulate appetite and reduce physical activity and metabolic rate during energy deficit – when combined with diet and exercise – would lead to better treatments for obesity.

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