Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight

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Abstract

Leptin can act as a satiety factor and exert neuroendocrine effects, but most studies have been performed in fasted animals. We aimed to determine the effect of chronic under-nutrition on the response to a 3-day intracerebroventricular infusion of leptin with regard to food intake and the secretion of pituitary hormones. Ovariectomised ewes (n=6) had a mean (± s.e.m.) bodyweight of 56 ± 0.8 kg on a diet available ad libitum (ad lib) or 33.4 ± 1 kg on a restricted diet. The differential bodyweight was achieved by dietary means over a period of 6 months prior to the commencement of the study. Leptin (4 µg/b) or vehicle (artificial cerebrospinal fluid (aCSF)) was infused into the third cerebral ventricle for 3 days. Blood samples were taken prior to commencement and on day 3 of infusion for the assay of plasma hormone levels. The experiment was repeated one week later in a crossover design. Food intake and metabolic status were monitored daily. The luteinising hormone (LH) pulse amplitude was lower (P<0.05) but plasma growth hormone (GH) levels were higher (P<0.05) in the food-restricted animals. Plasma levels of glucose, lactate, insulin, urea and triglycerides were similar in the two groups but non-esterified fatty acid levels were higher (P<0.01) in the animals on an ad lib diet. Leptin reduced (P<0.05) food intake only in the animals fed an ad lib diet. Leptin increased (P<0.05) the secretion of LH in the food-restricted group only and increased (P<0.05) GH irrespective of bodyweight. In conclusion, leptin does not alter food intake in animals on a restricted diet but can increase the secretion of LH in the same animals. The treatment of leptin was not sufficient to reduce plasma GH levels in the food-restricted animals, suggesting that other factors or mechanisms must be involved in the regulation of this axis.

Introduction

Leptin is secreted primarily by adipocytes and is capable of acting on the brain to signal satiety (Campfield et al. 1995, Halas et al. 1995). Defects in the leptin signalling pathway, due to a mutation in either the obese gene or the gene encoding the leptin receptor, result in morbid obesity in humans (Montague et al. 1997, Clement et al. 1998, Strobel et al. 1998) and rodents (Zhang et al. 1994, Lee et al. 1996). There are only a small number of humans who are afflicted with a mutation in the obesity gene (Maffei et al. 1996, Niki et al. 1996, Carlsson et al. 1997) and the role of leptin in normal physiology remains to be fully defined.

In addition to regulating energy homeostasis, leptin may serve as a signal to the neuroendocrine system and evidence suggests that this hormone is capable of altering the secretion of hormones from the pituitary gland by either direct or indirect (via the hypothalamus) means. Rodents and humans with mutations in the obese gene suffer from a range of endocrine abnormalities that can be ameliorated or reversed with exogenous leptin treatment, demonstrating the importance of leptin in maintaining hormone secretion (Farooqi et al. 1999, Ahima et al. 2000).

The effect of leptin on the secretion of hormones in normally fed animals remains a point of contention with various studies producing conflicting results. In male rats, intracerebroventricular (i.c.v.) infusion of leptin for 3 days (Vuagnat et al. 1998) or i.c.v. injection (Carro et al. 1997) had no effect on the secretion of growth hormone (GH), whereas another study (Tannenbaum et al. 1998) showed...
that a 7-day i.c.v. infusion stimulated GH secretion. Leptin stimulated GH release in pre-pubertal gilts when administered as an i.c.v. injection (Barb et al. 1998), but we (Henry et al. 1999) found no effect on plasma GH levels (or the levels of other pituitary hormones) in normally fed ovariectomised ewes.

In fasted animals leptin can rectify various endocrine perturbations. In rodents (Ahima et al. 1996) and pre-pubertal monkeys (Finn et al. 1998) peripheral administration of leptin restores the down-regulation of the hypothalmo–pituitary–gonadal axis induced by fasting. Furthermore, peripherally administered leptin restores to normal the plasma levels of adrenocorticotrophin, corticosterone, and the thyroid hormones in fasted mice (Ahima et al. 1996). In situ hybridisation studies have indicated that leptin can blunt the decrease in pro-thyrotrophin–releasing hormone mRNA in fasted rats (Legradi et al. 1997) and with regard to GH secretion, i.c.v. injection of leptin reversed the inhibitory effect of fasting in male rats (Carro et al. 1997). In contrast, i.v. infusion of leptin to adult male monkeys did not reverse the fasting–induced alterations in the secretion of luteinising hormone (LH), GH, cortisol or testosterone (Lado-Abeal et al. 2000). In this later experiment, however, leptin treatment did not begin until 11 h after the onset of the fast. Collectively, these studies suggest that responses to leptin in fasting animals may be dependent upon age (prepubertal vs adult) and the point at which leptin is administered.

Chronic under–nutrition is characterised by reduced gonadotrophin secretion (Thomas et al. 1990, Henry et al. 2000) and elevated GH secretion (Thomas et al. 1990, Barker-Gibb & Clarke 1996, Henry et al. 2000). Unlike acute fasting, chronic under–nutrition is associated with severe reductions in bodyweight and the percentage body fat. If leptin could restore endocrine changes to normal in these animals, this would suggest that it signals peripheral metabolic status to the brain. The ‘adipostat’ theory (Zhang et al. 1994) predicts that this would be the case, i.e. exogenous leptin would ‘deceive’ the brain with respect to nutritional and metabolic status. The current study investigated the effect of leptin in long-term food-restricted ovariec-tomised ewes in regard to satiety, metabolism and endocrine status. The aim was to determine whether centrally administered leptin can overcome the hunger drive and reverse the endocrine perturbations induced by chronic under–nutrition.

Experimental design

Twelve adult Corriedale ewes were ovariectomised at least 1 month before the study and fitted with guide tubes in the third cerebral ventricle (3 V) as previously described (Barker-Gibb et al. 1995). In November, the animals were divided into two groups and fed either a maintenance (lucerne hay available ad libitum +1 kg/week lupin grain) (ad lib) or a restricted (lucerne hay 400–500 g/day) diet. The dietary supplement (lupin grain) was offered over the period when voluntary food intake is greatest (November–March) (Clarke et al. 2000). Animals were weighed at various time-points to monitor the effect of diet on bodyweight. The animals on the maintenance diet were allowed to gain weight to account for the expected reduction in bodyweight that occurs during Autumn (reducing day length) in these seasonal breeders (Clarke et al. 2000). Following the removal of the lupin grain, the type of diet was standardised to lucerne chaff in both groups but the food-restricted animals were limited to 400–500 g to maintain low bodyweight. At the time of experimentation the two groups had average bodyweights of 56 ± 0·8 kg (ad lib) and 33·4 ± 1·0 kg (food-restricted).

The infusion protocol was performed in a cross-over design so that each animal received both leptin and artificial cerebrospinal fluid (aCSF: 150 mM NaCl, 1·2 mM CaCl2, 1 mM MgCl2, 2·8 mM KCl) as vehicle. Recombinant human leptin was synthesised and purified as previously described (Henry et al. 1999), dissolved in 1 mM HCl to a concentration of 1 mg/ml and diluted to 4 µg/50 µl in aCSF. Both leptin and vehicle solutions were infused into the 3 V at a rate of 50 µl/h for 72 h. One external jugular vein was cannulated and kept patent with heparinised (50 units/ml) normal saline. Serial blood samples (6 ml) were taken every 10 min for 8 h (0800–1600 h) prior to commencement and on day 3 of infusion. After the initial infusion period, the animals entered a 7-day recovery period at the end of which the infusion and sampling protocol was repeated in a cross-over design. Daily blood samples were taken across the infusion period at 0900 h to define the metabolic status of the animals and the concentration of follicle-stimulating hormone (FSH), and serial blood samples were used to study the secretory profile of LH and GH.

Two weeks after the leptin infusion the animals were killed and subjected to body composition analysis. The animals were eviscerated and the abdominal fat weighed; in addition, dual energy X-ray absorptiometry (DXA) (QDR4500, Hologic Inc., Waltham, MA, USA) was performed on the carcass.

Materials and Methods

Ethics

This work was approved in advance by the Animal Experimentation Ethics Committees of Monash University and Victoria Institute of Animal Science.

Radioimmunoassays

For LH analysis plasma samples (100 µl) were assayed in duplicate using the method of Lee et al. (1976). The standard used was ovine NIH-oLH-S18. For twelve assays...
the average sensitivity was 0·1 ng/ml, the intra-assay coefficient of variation (CV) was <10% over the range 0·8–26 ng/ml and the inter-assay CV was 7·5%.

For FSH analysis all samples (100 µl) were assayed in duplicate using the method of Brenner et al. (1980) with the ovine standard NIAMMD oFSH-RP-1. The sensitivity of the assay was 0·2 ng/ml and the intra-assay CV was <10% between 0·5–33 ng/ml.

For GH analysis plasma samples (200 µl) were assayed in duplicate using the method of Thomas et al. (1990) and the standard was NIDDK-oGH-I-4. For ten assays the average sensitivity was 0·2 ng/ml, the intra-assay CV was <10% over the range 2·6–38·6 ng/ml and the inter-assay CV was 16·9%.

Insulin was assayed in samples using a kit from Linco (St Louis, MO, USA) with human insulin as a standard and validated for ovine insulin in our laboratory (Henry et al. 1999). All insulin assays were conducted in a single assay with an intra-assay CV of 6·1%.

Plasma metabolites analyses: plasma non-esterified fatty acids (NEFA) were analysed using an enzymatic kit assay (Wako, Dallas, TX, USA) as outlined by Sechen et al. (1990). Plasma glucose, urea, triglycerides and lactate were measured using kit assays (Sigma Chemical Co., St Louis, MO, USA; procedures 510A, 640-B, 342–25P, 730–10 respectively). Sample and reagent volumes for the NEFA, glucose, triglycerides and lactate assays were modified to allow the assays to be conducted in ELISA plates and absorbances to be measured with an ELISA plate reader (Titertek Mutiskan MCC, Eflab, Finland). Inter- and intra-assay coefficients of variation were 5·8 and 4·7%, 2·9 and 1·5%, 3·9 and 2·6%, 4·5 and 3·7% and 2·5 and 1·3% for NEFA, glucose, urea, triglycerides and lactate respectively.

Data analysis
All body composition data are presented as a percentage in relation to the live bodyweight. The secretory profiles of LH and GH were characterised using pulse analysis techniques. LH pulses were calculated as previously outlined by Clarke (1993) and the TURBOPULSAR program was used to define GH pulses using the parameters described by Henry et al. (1999). To determine the effect of leptin on plasma metabolites and insulin levels and on the secretion of LH, GH and FSH, all data were corrected with respect to the basal levels and are therefore presented as the ratio of control to treatment periods.

Statistical analysis
Direct comparisons between normal and food-restricted animals for body composition, metabolic and endocrine data were performed using a single factor ANOVA. All data were checked for homogeneity of variance; abdominal fat mass, urea, insulin, NEFA, lactate, triglycerides, LH pulse amplitude and GH mean concentration were subjected to log transformation. The effect of diet on bodyweight, and leptin treatment on food intake were performed by repeated measures ANOVA. The analyses of the effect of leptin on the secretion of LH, FSH and GH were performed using a repeated measures ANOVA incorporating treatment, group and order effects. Prior to analysis the LH inter-pulse interval, pulse amplitude, mean concentration and pre-pulse nadir, and the GH pulse amplitude were subjected to log transformation. Where appropriate, paired comparisons were made using least significant differences.

Results

Bodyweight and body composition analysis (Fig. 1)
The bodyweights of both groups had significantly (P<0·01) diverged within 1 month of dietary manipulation. At the time of the leptin infusion (May) the two groups had significantly (P<0·001) different bodyweights with the ad lib and food-restricted animals weighing 56 ± 0·8 kg and 33·4 ± 1 kg respectively. Bodyweights in the food-restricted animals stabilised; no further reduction in bodyweight occurred after April. Body composition analysis expressed as the percentage of bodyweight (g) showed that food-restricted animals had lower (P<0·01) total body and abdominal fat mass. The lean body mass and the bone mineral content was similar in the ad lib and the food-restricted animals.

Metabolite and hormone levels in ad lib and food-restricted animals (Table 1)
The mean plasma levels, inter-pulse interval and pre-pulse nadir for LH secretion were similar in ad lib and food-restricted animals, whereas the pulse amplitude was lower (P<0·05) in the food-restricted group. Mean plasma growth hormone levels and baseline concentrations were higher (P<0·05) in the food-restricted group. In addition, the GH inter-pulse interval was lower (P<0·05) in the food-restricted group but the pulse amplitude was similar in ad lib and food-restricted animals. Plasma FSH levels were similar in ad lib and food-restricted animals. Plasma urea, insulin, glucose, lactate and triglyceride concentrations were also similar in ad lib and food-restricted animals but plasma NEFA concentrations were lower (P<0·01) in the food-restricted group.

The effect of leptin on voluntary food intake in ad lib and food-restricted animals (Fig. 2)
Food intake was similar in the ad lib and food-restricted animals across the experimental period, despite the fact that the food-restricted animals were limited to
500 g per day. Leptin treatment reduced \( P < 0.05 \) food intake in the ad lib animals only. This effect was still apparent 2 days post-infusion.

The effect of leptin on plasma metabolites and hormone levels in ad lib and food-restricted sheep (Figs 3–6)

There was no effect of the 3-day leptin infusion on the plasma levels of glucose, lactate, urea, insulin and tri-glycerides but leptin treatment increased \( P < 0.05 \) plasma NEFA concentrations in the ad lib animals (Fig. 3). The effect of leptin on plasma NEFA levels was more apparent when leptin was administered in the second week of the experiment compared with the first. Leptin treatment partially restored \( P < 0.05 \) the plasma levels of LH in the food-restricted group predominantly due to an elevation \( P < 0.05 \) in the pre-pulse nadir although there was a trend towards a decrease \( P = 0.068 \) in the inter-pulse interval.

Table 1 The effect of nutritional status on the mean \( \pm \text{S.E.M.} \) plasma hormone and metabolite levels

<table>
<thead>
<tr>
<th></th>
<th>Ad lib</th>
<th>Food restricted</th>
<th>( \text{P} &lt; 0.05 )</th>
<th>( \text{P} &lt; 0.01 )</th>
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<tbody>
<tr>
<td>LH mean concentration (ng/ml)</td>
<td>5.7 ± 1.3</td>
<td>3.3 ± 0.9</td>
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<tr>
<td>LH pulse amplitude (ng/ml)</td>
<td>3.5 ± 0.6</td>
<td>2.0 ± 0.2</td>
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<tr>
<td>LH inter-pulse interval (min)</td>
<td>82.1 ± 31.9</td>
<td>64.5 ± 12.6</td>
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<tr>
<td>LH pre-pulse nadir (ng/ml)</td>
<td>4.2 ± 0.9</td>
<td>2.6 ± 0.8</td>
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<tr>
<td>GH mean concentration (ng/ml)</td>
<td>8.0 ± 1.9</td>
<td>23.0 ± 6.9</td>
<td></td>
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<tr>
<td>GH pulse amplitude (ng/ml)</td>
<td>9.1 ± 2.0</td>
<td>19.5 ± 7.3</td>
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<tr>
<td>GH inter-pulse interval (min)</td>
<td>49.1 ± 4.0</td>
<td>38.9 ± 2.9</td>
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<tr>
<td>GH baseline concentration (ng/ml)</td>
<td>5.2 ± 1.2</td>
<td>17.3 ± 5.2</td>
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<tr>
<td>FSH (ng/ml)</td>
<td>12.6 ± 2.2</td>
<td>16.0 ± 1.0</td>
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<tr>
<td>Glucose (mM)</td>
<td>3.5 ± 0.2</td>
<td>3.4 ± 0.1</td>
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<tr>
<td>Lactate (mM)</td>
<td>2.0 ± 0.4</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>Insulin (µU/ml)</td>
<td>140.0 ± 4.0</td>
<td>6.5 ± 1.2</td>
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<tr>
<td>Urea (mM)</td>
<td>5.2 ± 0.5</td>
<td>4.3 ± 0.2</td>
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<tr>
<td>NEFA (µM)</td>
<td>687 ± 122</td>
<td>350 ± 14.6</td>
<td></td>
<td></td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.2 ± 0.04</td>
<td>0.1 ± 0.02</td>
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</table>
Leptin exerted a greater influence on the secretion of LH in the food-restricted animals in the first week of the experiment (Fig. 5). There was no effect of the 3-day leptin infusion on any parameter of LH secretion in the ad lib sheep. The mean concentration of GH was increased ($P < 0.05$) by approximately 25% in both ad lib and food-restricted sheep throughout leptin treatment (Fig. 4). There was no effect of leptin on the inter-pulse interval or baseline concentration in either group, the main effect was to increase GH pulse amplitude ($P < 0.05$) and this occurred in the ad lib animals only (Fig. 4). This effect was predominant throughout the second week of treatment (Fig. 6). There was no effect of leptin infusion on the mean plasma concentrations of FSH in either ad lib or food-restricted sheep (data not shown).

**Discussion**

This study shows that long-term alterations in nutritional status influence the response to a 3-day i.c.v. infusion of leptin with regard to both appetite and the secretion of LH. Many studies have shown leptin to be capable of reversing the endocrine perturbations caused by short-term fasting (discussed below), which is an acute metabolic stressor, but this is the first time leptin has been shown to increase the secretion of LH in animals that have been chronically food-restricted. In addition, leptin treatment had differential effects on appetite in ad lib and food-restricted sheep; leptin inhibited food intake in the ad lib animals only, suggesting that the hunger drive in animals during food restriction is not counter-acted by leptin. The secretion of GH was consistently increased by the infusion of leptin and this effect was irrespective of bodyweight, indicating that leptin cannot decrease the high levels of GH in food-restricted animals via central networks.

It was most interesting that leptin could not reduce food intake in the food-restricted group below the limited amount of 500 g/day. Previous in situ hybridisation studies have shown that long-term food restriction increases the expression of the orexigenic peptide neuropeptide Y (NPY) in ewe lambs (McShane et al. 1993) and in gonadectomised sheep (Adam et al. 1997, Henry et al. 2000). Expression of another orexigenic peptide, melanin-concentrating hormone (MCH), is also increased in under-fed ovariectomised ewes (Henry et al. 2000). In addition, immunocytochemical techniques have shown increased peptide levels of NPY in under-fed ovariectomised ewes (Barker-Gibb & Clarke 1996). We take this increase in both the expression of genes and peptide levels for orexigenic factors to reflect hunger in these animals. Although these factors were not measured in the current study (because of the cross-over design), we have previously shown that leptin decreases NPY mRNA levels in normally fed animals (Henry et al. 1999). It is therefore reasonable to presume that the food-restricted animals of the current study would have had increased NPY and/or MCH levels. Given that leptin treatment could not reduce food intake in these food-restricted animals, it is most likely that the treatment could not correct the NPY and/or MCH levels towards normal, but this hypothesis remains to be tested. The food-restricted animals were
indeed hungry, as when offered an ad lib diet the voluntary food intake of the food-restricted group almost doubled (919 ± 9 g/day) (I J Clarke, unpublished data). This suggests that, although the animals were stable in regard to both metabolism and bodyweight (see below), they were in a state of non-satiety, and leptin (4 µg/h for 3 days) was not sufficient to overcome this. It would be interesting to determine whether leptin could affect the refeeding response in these animals, but this was not possible in the current experimental design. Because we adopted a cross-over design, offering an ad lib diet to the food-restricted animals was not appropriate because it would have perturbed their ‘steady-state’ metabolic condition in the first half of the experiment and this would have compromised the second half. Foster et al. (1989) have shown that feeding the growth-limited female lamb an ad lib diet induces an LH pulse within 1 h and LH secretion is increased within 24 h. Feeding the food-restricted animals an ad lib diet would, therefore, have prevented us from dissociating any effect of leptin per se from an increase in caloric intake on endocrine status.

Neuroendocrine function was altered in accordance to the changes in nutritional status. The effect of nutritional status on the secretion of LH is dependent on the time and extent of under nutrition. Previously, chronic food restriction has been found to reduce the LH inter-pulse interval (Foster & Olster 1985, Thomas et al. 1990), to lower the mean concentration (Henry et al. 2000) or to have no

Figure 3 The effect of i.c.v. infusion of aCSF or leptin (4 µg/h for 3 days) on the metabolic state of the animals indicated by mean (± S.E.M.) plasma concentrations of urea, insulin, glucose, lactate, non-esterified fatty acids (NEFA) and triglycerides. Open bars represent food-restricted animals and solid bars the ad lib group. All data were corrected with respect to pre-treatment values (=1). *P<0.05, leptin compared with aCSF.

Figure 4 The effect of i.c.v. infusion of aCSF or leptin (4 µg/h for 3 days) on the secretory profile of LH and GH. The secretory profile was assessed by analysis of the over-all mean concentration (mean), and the mean values for the pulse amplitude (Amp), the inter-pulse interval (IPI) and either the pre-pulse nadir (Nadir) or the baseline concentration (Baseline). All data were corrected with respect to pre-treatment values (=1) and are presented as the mean (± S.E.M.). Open bars represent data from the food-restricted animals and solid bars represent data from the ad lib animals. *P<0.05, leptin compared with aCSF.

Figure 5 Representative secretory profiles for plasma luteinising hormone (LH) concentrations in animals fed either an ad lib or a food-restricted diet when treated with leptin (4 μg/h for 72 h) or artificial aCSF. Infusion and blood sampling was performed in a cross-over design so that each animal received both leptin and vehicle (aCSF). Part I represents the first week of experimentation and Part II the second week after the cross-over design had been incorporated. Note that leptin treatment increased the pre-pulse nadir in the food-restricted animals (sheep #147 and #180) and this effect was predominant in the first week of the experiment. Leptin treatment did not alter the secretory profile for LH in the animals on an ad lib diet (sheep #128 and #207).
Figure 6  Representative secretory profiles for plasma growth hormone (GH) concentrations in animals fed either an ad lib or a food-restricted diet when treated with leptin (4 μg/h for 72 h) or aCSF. Infusion and blood sampling was performed in a cross-over design so that each animal received both leptin and vehicle (aCSF). Part I represents the first week of experimentation and Part II the second week after the cross-over design had been incorporated. Note that leptin treatment increased GH in the food-restricted and ad lib animals, an effect that was greatest in the second week (Part II) of the experiment as seen in sheep #180 and #42.
effect on any LH parameter (Barker-Gibb & Clarke 1996). In one study, the mean plasma concentration of LH increased in food-restricted castrated sheep due to an increase in pulse amplitude, despite a reduction in the inter-pulse interval (Adam et al. 1997). In the current study, the LH pulse amplitude was lower and the mean concentration of GH was higher in the food-restricted animals. In addition to the effects of leptin on food intake, we have shown that leptin is able partially to restore the secretion of LH in the food-restricted sheep. The mean concentrations and the pre-pulse nadir were increased by the infusion of leptin in food-restricted animals and there was a trend towards reduced inter-pulse interval (increased pulse frequency). Intuitively, an increase in the pre-pulse nadir is an indication of reduced inter-pulse interval. This observation is similar to responses in fasted monkeys (Finn et al. 1998) and rodents (Ahima et al. 1996, Nagatani et al. 1998), where peripheral administration of leptin has been shown to correct lowered plasma LH levels. The effect of leptin on the secretion of LH was greater in the first part of the experiment and this may be attributable to an improvement in the housing conditions of the animals throughout the experiment and therefore a slight correction in the secretion of LH. It has previously been shown that leptin does not stimulate the secretion of LH in animals with a normal secretory profile and this could account for the attenuated response to leptin in the second half of the experiment (Henry et al. 1999).

Mean GH concentrations were increased by approximately 25% after a 3-day infusion of leptin in both ad lib and food-restricted sheep. This is consistent with previous studies in male rats (Tannenbaum et al. 1998) and pre-pubertal gilts (Barb et al. 1998) where i.c.v. infusion or injection respectively have been shown to increase GH secretion. On the other hand, we have previously reported a lack of effect of leptin on plasma GH levels in normally fed ovariectomized ewes using a range of doses (1 µg/h–20 µg/h) or a variable time-span (3 days–1 week) (Henry et al. 1999, I J Clarke, unpublished data). The effect of leptin in increasing GH secretion is not consistent with the supposed role of leptin as an ‘adipostat’ but corresponds to the reduction in food intake seen in the ad lib group, since plasma GH levels are generally increased with fasting (Driver & Forbes 1981). On the other hand, leptin increased GH levels in ad lib and food-restricted animals alike, thus dissociating the effects of leptin per se and reduced food intake. We must therefore conclude that the central effect of leptin is to stimulate GH secretion. In fasted rodents, the effect of leptin on GH secretion can be blocked by growth hormone-releasing hormone (GHRH) antiserum (Carro et al. 1999) indicating that, at least in this species, the hypothalamus is important in mediating the effect of nutritional alterations in GH secretion. It is also noteworthy that the effects of leptin on GH secretion in the present study were much less than those obtained in gilts with a single 3 V injection (Barb et al. 1998).

Although the present study was not designed to monitor acute effects, we have not seen an acute response to leptin injection in sheep (I J Clarke, unpublished data). Data from this laboratory (Roh et al. 1998) show a negative effect of leptin on GHRH-stimulated GH secretion from somatotrophs in primary cultures and a large percentage (69%) of somatotrophs possess the leptin receptor (Iqbal et al. 2000). It is possible that higher levels of leptin in ad lib animals act in a negative manner to regulate GH secretion at the level of the pituitary gland, leading to lower circulating levels of GH in this condition.

In our current experiments, the mean concentration of FSH was similar in the ad lib and food-restricted animals, but altered nutritional status can exert variable effects on the secretion of FSH in adult animals. High protein supplements like lupin grain and cowpea have been shown to stimulate the secretion of FSH (Pomares et al. 1995), whereas restricted nutrition induced either a small reduction (Thomas et al. 1990) or no change in FSH levels (Barker-Gibb & Clarke 1996). The present data are consistent with our previous results where leptin did not influence FSH secretion in normally fed ovariectomised ewes (Henry et al. 1999).

The current study employed a central infusion to determine whether leptin could have a direct effect on the neuroendocrine centres in the hypothalamus regulating LH secretion. Potentially, leptin may affect the gonadotrophin-releasing hormone (GnRH) neurons either directly or indirectly via a second neuronal system, e.g. MCH or orexin. Melanin-concentrating hormone stimulates the secretion of LH in anaethetised rats (Gonzalez et al. 1997, Murray et al. 2000) but inhibits LH release in normal animals (Tsukamura et al. 2000), whereas orexin inhibits LH secretion in ovariectomised rats (Pu et al. 1998, Tamura et al. 1999). It would seem unlikely that leptin would have a direct effect on GnRH neurons, since these cells express either low leptin receptor levels or no receptors in rodents (Hakansson et al. 1998) and there is no co-localisation of GnRH and leptin receptor in monkeys (Finn et al. 1998). The extent of leptin receptor and GnRH co-localisation in the sheep brain remains unknown. It is possible, therefore, to invoke a mechanism whereby chronic under-nutrition increases the levels of orexigenic peptides such as NPY and MCH which inhibit LH secretion (Barker-Gibb et al. 1995, Tsukamura et al. 2000). Leptin treatment corrects the altered peptide levels to a point where GnRH/LH release is increased but restricted food intake is not altered.

The plasma concentrations of glucose, lactate, urea, insulin and triglycerides were similar in ad lib and food-restricted animals but NEFA levels were higher in the ad lib group. This demonstrates that both groups were metabolically stable and blood sugar levels were maintained within the normal range despite the difference in bodyweight. The elevated NEFA levels in the ad lib animals can be considered a reflection of the higher body
fat content (Baile & Forbes 1974), as indicated by body composition analysis (see below). The 3-day infusion of leptin had no effect on most plasma metabolites but did increase the NEFA levels in the ad lib group. This indicates the mobilisation of body stores in response to the inhibition of food intake (Baile & Forbes 1974), supporting earlier findings from this laboratory (Henry et al. 1999). The results obtained from the body composition analysis reflected long-term alterations in adiposity. The percentage of total body fat content and the percentage of abdominal fat mass were both lower in the food-restricted animals but the lean body mass and bone mineral content were similar in the two groups. These data further demonstrate that the food-restricted animals are metabolically stable, as there was no deterioration of lean tissue as evidenced by maintenance of lean body mass, nor was there any deterioration in the bone mineral content. Although the animals fed an ad lib diet gained weight and had higher adiposity than those on the restricted diet they cannot be considered obese. There was no indication of elevated plasma insulin levels consistent with insulin-resistance (Bergman et al. 1989) nor was there any evidence for lowered percentage lean body mass, both parameters being characteristic traits of obesity in sheep (McCann et al. 1992, Henry et al. 2000).

In conclusion, we have shown that long-term changes in nutritional status influence the response to a 3-day i.c.v. infusion of leptin with regard to both food intake and the secretion of LH. Leptin treatment does not over-ride the hunger drive in chronically food-restricted animals to a point where the limited food intake is altered but it can act to increase LH in long-term food-restricted animals. Leptin does not appear to act as an ‘adipostat’ at the level of the hypothalamus with regard to GH secretion as this was increased in both the ad lib and food-restricted groups. The current study shows that the effects of leptin on neuroendocrine status and food intake are dissociated in ovariectomised ewes.

Acknowledgements

We thank Mr Bruce Doughton and Ms Karen Perkins for animal care, Ms Alexandra Rao for performing most of the LH assays, Prince Henry’s Institute of Medical Research for a stipend to support B A H, and Mr David Caddy for advice on statistical analyses. Hormone reagents and standards were supplied by National Hormone and Pituitary Program of NIDDKD. The work was supported by The National Health and Medical Research Council of Australia.

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Received 7 August 2000
Revised manuscript received 11 September 2000
Accepted 20 September 2000

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