CLINICAL STUDY

Hyperleptinemia in women with Cushing's disease is driven by high-amplitude pulsatile, but orderly and eurhythmic, leptin secretion

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Abstract

The episodicity of 24 h leptin release was studied in seven women (mean age 39 years, range 22–56 years) with pituitary-dependent hypercortisolism and in seven age- and body mass index (BMI)-matched female controls. Pulsatile leptin release was quantified by model-free CLUSTER analysis and deconvolution, the orderliness of leptin patterns by the approximate entropy statistic (ApEn), and nyctohemeral leptin rhythmicity by cosinor analysis. Blood samples were taken at 10 min intervals for 24 h. Both CLUSTER and deconvolution analysis revealed 2.4-fold increased leptin secretion in patients, caused by combined and equal amplification of basal and pulsatile secretion. Cluster analysis identified 7.1 ± 1.5 peaks per 24 h in patients and 6.0 ± 0.5 in controls (not significant). The statistical distribution of the individual sample secretory rates was similarly skewed in patients and controls (0.55 ± 0.12 vs 0.52 ± 0.07). The acrophase (timing of the nyctohemeral leptin peak) in patients occurred at 2314 h (± 76 min) and at 0058 h (± 18 min) in controls (not significant). The approximate entropy of leptin release was equivalent in patients and controls (1.67 ± 0.03 vs 1.61 ± 0.05). The approximate entropy (ApEn) for cortisol in patients was 1.53 ± 0.09 and in controls was 0.93 ± 0.07 ($P < 0.0005$). Cross-ApEn showed significant pattern synchrony between leptin and cortisol release, which (unexpectedly) was not disrupted by the cortisol excess (patients, 2.02 ± 0.04 ; controls, 1.88 ± 0.09 ; $P = 0.233$). Insulin levels in fasting patients ('fasting insulin') were 27 ± 5.7 mU/l vs 14 ± 1.6 mU/l in controls ($P = 0.035$). Leptin secretion correlated with fasting insulin levels ($R^2 = 0.34$, $P = 0.028$) and with the cortisol production rate ($R^2 = 0.33$, $P = 0.033$) when patients and controls were combined. In summary, Cushing's disease in women increases leptin production about twofold in an amplitude-specific way. The pulse-generating, nyctohemeral phase-determining, and entropy-control mechanisms that govern the 24 h leptin release are not altered. The increased secretion is not explained by BMI and is probably only partly explained by increased insulin production, suggesting a cortisol-dependent change in adipose leptin secretion.

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Introduction

Leptin, the protein product of the *ob* gene, is produced and secreted primarily by adipose tissue and in smaller amounts by the placenta and central nervous system (1). This metabotropic protein binds to specific receptors belonging to the cytokine receptor family (2), which are expressed in many tissues, including that of brain and hypothalamus, thereby conveying feedback information on the quantity of energy stored. Plasma leptin levels are positively correlated with the body mass index (BMI), the percentage of body fat (3), and adipocyte cell size (4). Leptin secretion is higher in premenopausal and postmenopausal females than in comparably aged males (5), at any given level of

adiposity (6). This gender difference emerges after the onset of puberty (7). Accordingly, it could be hypothesized that gonadal steroids are involved in the regulation of leptin production. In addition, *in vitro* and *in vivo* studies in experimental animals and humans have demonstrated that acute administration of glucocorticoids stimulates leptin gene transcription and increases plasma leptin concentrations (8–11). In Cushing's syndrome, mean plasma leptin levels, collected hourly over 24 h, were elevated twofold compared with values in BMI-matched controls (12), and 'fasting leptin' was increased fourfold compared with lean controls (13). However, the nature of leptin's episodic release or diurnal regulation in Cushing's disease remains unknown. Such information would probably offer

further insights into the putative relationship between sustained endogenous hypercortisolemia and altered control of leptin production in the adult with Cushing's syndrome.

Subjects and methods

Subjects

Seven women with pituitary-dependent Cushing's disease (mean age, 39 years; range, 22–56 years) and seven age-, BMI- and gender-matched healthy controls (mean age, 44 years; range, 32–58 years) were studied. The diagnosis was established by elevated 24 h urinary excretion of free cortisol, subnormal suppression of urinary cortisol excretion during a low-dose dexamethasone test, suppression of plasma cortisol concentration by 190 nmol/l or more during a 7 h i.v. infusion of dexamethasone at a dose of 1 mg/h (14), positive immunostaining of the adenoma, and cortisol-dependency for at least three months post-operatively. The mean urinary excretion of free cortisol was 864 nmol/24 h (range, 241–2040, normal range, 40–200). All patients and control subjects were clinically and biochemically euthyroid and were not using any medication. Premenopausal controls were studied in the early follicular phase of the menstrual cycle.

Methods

Patients and control subjects were admitted to the hospital on the day of the study. An indwelling i.v. cannula was inserted in a forearm vein 60 min before the start of sampling, and blood samples were withdrawn at 10 min intervals and for the next 24 h, starting at 0900 h. The subjects were free to ambulate, but not to sleep, during the day. Meals were served at 0800, 1230 and 1730 h. Lights were turned off between 2200 and 2400 h. Plasma samples were collected on ice in heparinized tubes and centrifuged at 4 °C for 30 min. The plasma was frozen at –20 °C until later assay. Informed consent was obtained from all patients and control subjects, as approved by the Ethical Committee of the Leiden University Medical Center.

Assays

Leptin was measured by radioimmunoassay (RIA) (Linco Research, St Charles, MO, USA), with a detection limit of 0.5 µg/l and an intra-assay coefficient of variation of 3.4–8.3% over the leptin concentration range of 4.9–25.6 µg/l; the corresponding interassay coefficient of variation was 3.0–6.2%. Cortisol was assayed by RIA (Sorin Biomedica, Milan, Italy) with a detection limit of sensitivity of 25 nmol/l. The intra- and interassay precision varied from 2 to 4%. Growth

hormone (GH) was measured by time-resolved fluoro-immunoassay (Wallac, Turku, Finland) specific for the 22 kDa GH, which was used as the standard (Genotropin; Pharmacia Upjohn, Uppsala, Sweden) as calibrated against the World Health Organization First International Reference Preparation, 80/505 (to convert µg/l to mU/l, multiply by 2.6). The limit of detection was 0.0115 mU/l. The intra-assay coefficient of variation varied from 1.6 to 8.4% in the assay range from 0.26 to 47 mU/l and the interassay coefficient of variation was 2.0–9.9% in the same range. Insulin was assayed by RIA with a detection limit of 3.6 mU/l. The interassay coefficient of variation was 3.8–8.0% over the concentration range of 12.5–94.5 mU/l. All samples from one individual were run in the same batch.

Analytical techniques

CLUSTER, a largely model-free computerized peak-detection algorithm, was used to quantify statistically significant pulses in relation to dose-dependent measurement error in a serum leptin concentration vs time series (15). A 2 × 2 test cluster size was used to test for rises and falls in the data and *t* statistics of 2.0 for significant up- and down-strokes for leptin peaks. CLUSTER analysis was validated to quantify the pulsatile properties of leptin release in earlier studies (16, 17). A waveform-independent deconvolution analysis (PULSE) was used to calculate the mean and nadir 24 h leptin secretion (18, 19), based on the published one-component half-life for leptin of 24.9 min (20). Cortisol and GH secretion parameters were estimated by multi-parameter deconvolution analysis (18). The diurnal rhythm of plasma leptin concentrations was appraised by cosinor analysis. Cosinor analysis simply entails trigonometric regression of a cosine function on the full 24 h plasma hormone concentration profile vs time.

ApEn was used as a scale- and model-independent statistic to quantify the serial orderliness or regularity of leptin release over 24 h. Normalized ApEn parameters of $m = 1$ and $r = 20\%$ of the intraseries s.d. were used, as previously described (21). Hence, ApEn is designated ApEn(1,20%), which quantifies the regularity of subordinate (non-pulsatile) in the data. ApEn yields information complementary to cosinor and peak analyses or deconvolution techniques. Cross-ApEn evaluates relative pattern orderliness between two hormone series (22).

Statistical analysis

Statistical analysis was performed using SPSS for Windows (Release 8.0; SPSS, Chicago, IL, USA). Results are expressed as the means ± s.e.m. The Student's paired, two-tailed *t*-test was used to compare leptin concentrations between groups. Differences were considered significant for $P < 0.05$. Linear regression

Figure 1 Individual 24 h plasma leptin concentration profiles in two patients with pituitary-dependent Cushing's disease and their respective controls. Note the difference in scale between patients and controls.

analysis was performed to analyse the relationships between leptin and insulin or cortisol production. Data were transformed logarithmically for comparisons of derived values.

Results

Figure 1 shows the individual 24 h plasma leptin concentration profiles of two patients and their respective

controls. The BMI was $26.4 \pm 1.3 \text{ kg/m}^2$ in patients and $24.5 \pm 1.6 \text{ kg/m}^2$ in controls ($P = 0.167$). Mean and integrated plasma leptin concentrations were more than twofold higher in patients than in controls (see Table 1). This increase was accounted for by an elevation in both the interpeak nadir concentration and incremental peak height (see Fig. 2).

The mean calculated leptin secretion rate was $0.772 \pm 0.135 \text{ µg/l per min}$ in patients and $0.333 \pm 0.052 \text{ µg/l per min}$ in controls ($P = 0.004$). Nadir

Table 1 Cluster analysis of leptin secretion in patients with Cushing's disease and in controls. Values are expressed as means $\pm$ S.E.M. Blood samples were withdrawn at 10 min intervals for 24 h in seven women with Cushing's disease and seven control women matched for age and body mass index. Significance was determined using the paired two-tailed Student's *t*-test.

Parameter	Patients	Controls	<i>P</i> value
Integrated concentration (µg/l/24 h)	$45\,700 \pm 7900$	$19\,700 \pm 3100$	0.007
No. of peaks	7.1 ± 1.5	6.0 ± 0.5	NS*
Peak width (min)	131 ± 35	154 ± 27	NS*
Peak area (µg/l/min)	658 ± 106	392 ± 61	NS*

* NS, not significant.

Figure 2 Comparisons of mean 24 h plasma leptin concentrations (a), maximal leptin peak heights (b), interpeak nadir values (c) and incremental leptin peak elevations (d) in seven women with Cushing's disease and seven BMI-matched controls. *P* values were determined as in Table 1.

secretion rates were $0.682 \pm 0.119 \mu\text{g/l per min}$ in patients and $0.292 \pm 0.045 \mu\text{g/l per min}$ in controls ($P = 0.004$). Table 2 gives the 24 h secretion rates for leptin, cortisol and GH, and shows significantly higher secretion rates for leptin and cortisol, but not for GH, in patients compared with matched controls. The statistical distributions of the 10 min sample secretion rates were similar in patients and controls. The skewness

Table 2 Daily secretion rates for leptin, GH and cortisol, and fasting plasma insulin concentrations in female patients with Cushing's disease and in matched controls. Values are the means $\pm$ S.E.M. and are expressed as mass units per liter distribution volume. Blood samples were withdrawn at 10 min intervals for 24 h in seven women with Cushing's disease and seven control women, matched for age and body mass index. The mean plasma insulin concentration was the mean of six early morning fasting samples. The significance was determined using the paired two-tailed Student's *t*-test.

Parameter	Patients	Controls	<i>P</i> value
Leptin secretion ($\mu\text{g/l}$)	1112 ± 194	480 ± 75	0.007
GH (mU/l)	515 ± 91	568 ± 108	0.747
Cortisol ($\mu\text{mol/l}$)	116 ± 12	51 ± 9	0.001
Insulin (mU/l)	26.9 ± 5.7	13.6 ± 1.6	0.035

was 0.55 ± 0.12 in patients vs 0.52 ± 0.07 in controls ($P = 0.885$), with similar kurtosis estimates of, respectively, 0.30 ± 0.32 vs 0.11 ± 0.16 in patients and controls ($P = 0.64$). Thus, mean and nadir leptin secretion rates were elevated in Cushing's disease, without any distortion of their relationships. Cosinor analysis disclosed significant differences in the 24 h rhythmic mean plasma leptin concentration (patients, $31.8 \pm 5.5 \mu\text{g/l}$; controls, $13.7 \pm 2.2 \mu\text{g/l}$; $P = 0.007$). The fractional amplitude of the daily leptin rhythm was similar in the two groups (patients, 0.17 ± 0.03 ; controls, 0.23 ± 0.04 ; $P = 0.331$). In addition, patients and controls exhibited comparable acrophases (patients, $2314 \text{ h} \pm 76 \text{ min}$; controls, $0058 \text{ h} \pm 18 \text{ min}$; $P = 0.181$).

The regularity of leptin secretion, as measured by ApEn (1.20%), did not differ between patients and controls: 1.67 ± 0.03 vs 1.61 ± 0.05 respectively ($P = 0.393$). Synchrony quantification by Cross-ApEn of the cortisol–leptin time series revealed respective values of 2.02 ± 0.04 in patients and 1.88 ± 0.09 in controls ($P = 0.223$). In contrast, the ApEn (1.20%) for cortisol was elevated at 1.53 ± 0.09 in patients vs 0.93 ± 0.07 in controls ($P < 0.0005$). This contrast denotes a markedly disorderly cortisol release in patients.

Fasting plasma insulin concentrations (mean of six consecutive early morning samples) were 50% lower in controls than in patients (13.6 ± 1.6 mU/l vs 26.9 ± 5.7 mU/l respectively; $P = 0.035$).

In patients, no significant correlations were found between the 24 h cortisol-production and leptin-pulse parameters, leptin secretion or leptin ApEn. Pooling of data from patients and controls revealed significant correlations between the 24 h leptin secretion rate and fasting plasma insulin concentrations ($R^2 = 0.34$; $P = 0.028$) and 24 h cortisol production ($R^2 = 0.33$; $P = 0.033$), but not 24 h GH production ($R^2 = 0.074$, $P = 0.348$).

Discussion

Women with pituitary-dependent Cushing's disease exhibit a more than twofold elevation in the daily rate of leptin secretion compared with age-, gender- and BMI-matched controls, as assessed here by two independent analytical techniques. Discrete peak-detection analysis demonstrated proportionate increases in the interpeak nadir serum leptin concentration and incremental leptin peak amplitude. The number of plasma leptin concentration peaks per 24 h did not differ between groups, thus excluding an increase in pulse frequency as the driving mechanism for relative hyperleptinemia. The present analyses corroborate and extend the results reported by Leal-Cerro *et al.*, who utilized hourly blood sampling (12). The latter study could not include detailed pulse and entropy evaluation, but also identified a nearly twofold elevation of mean plasma leptin in Cushing's syndrome compared with eucortisolemia. As weight gain is a frequent clinical sign of Cushing's disease (23), the increased plasma leptin concentration might be interpreted as a physiological adaptation that signals appetite restraint despite hypercortisolism. The observation that circulating leptin concentrations did not fall 10 days following correction of the hypercortisolemic state could further suggest that factors other than ongoing cortisol hypersecretion also play a significant role in leptin overproduction in established Cushing's syndrome (13).

Systemic leptin concentrations in healthy individuals typically correlate positively with BMI, percentage body fat and total body fat (6). Regional fat distribution has a predominant impact on circulating leptin levels, since subcutaneous (but not visceral) fat mass measured by computed tomography or magnetic resonance imaging predicts leptin concentrations (24, 25). Van Harmelen and co-workers demonstrated, by using direct biopsy measurements, that leptin secretion from subcutaneous fat was 2–3 times higher than that from omental fat, although both fat compartments contributed significantly to the circulating leptin level (26). Patients with Cushing's disease have reduced lean body mass as well as increased fat mass compared with normal subjects. However, a recent detailed comparative study, by

Wajchenberg *et al.*, of patients with Cushing's disease and BMI-matched healthy controls showed that total body fat is no higher, but that the proportion of visceral fat to subcutaneous truncal fat is increased by 60% (27). This topography corresponds to an apparent shift of about 1.6 kg from subcutaneous to visceral abdominal fat stores. Although we do not have body compositional data for our patients, assuming analogous distribution, we reason that preferential omental fat accumulation in Cushing's syndrome is not an evident explanation for the hyperleptinemia observed in this hypercortisolemic disease.

In contrast to the positive association between fat stores and leptin production, lean body mass is a negative statistical determinant of plasma leptin levels (5, 28, 29). In this regard, Wajchenberg *et al.* quantified slightly lower absolute (but not fractional) lean body mass in Cushing's disease compared with obese controls. Accordingly, the role of altered lean body mass in hyperleptinemia associated with Cushing's syndrome is not yet clear.

GH secretion is negatively correlated with total body fat and, in particular, central obesity, as well as with leptin secretion. GH, but not insulin-like growth factor, directly inhibits leptin gene expression in visceral fat in the obese rat (30). Likewise, treatment with recombinant human GH reduces plasma leptin levels in GH-deficient patients in parallel with a fall in fat mass and a rise in lean body mass (31, 32). Thus, it could be inferred that diminished GH secretion in Cushing's syndrome results in disinhibition of leptin secretion (33, 34). Indeed, patients with Cushing's disease evince a negative relationship between GH and cortisol secretion rates (35). Nonetheless, overtly subnormal GH secretion was only present in Cushing's patients with severe hypercortisolism. Thus, attenuation of GH secretion in Cushing's disease may be a plausible, but not exclusive, factor in the pathophysiology of hyperleptinemia in this disorder.

Chronic hyperinsulinemia also appears to stimulate leptin production (36–41). Insulin can heighten leptin secretion by human adipocytes *in vitro* (36). These data are consistent with, but not proof of, a causal relationship between hyperinsulinism and increased leptin production, as observed here jointly in Cushing's disease. Analogous reasoning has been applied to obese patients with polycystic ovary syndrome, although this relationship is not evident in all studies (42–44).

Rhythmic leptin secretion was similar in patients and controls. The genesis of the 24 h variations in leptin production is not well established, except that such rhythmicity is not caused exclusively or primarily by diurnal variations in energy intake (45). Inasmuch as fasting maintains the nyctohemeral rhythmicity of leptin synthesis and secretion as modulated by ambient putative neural signals, our results tend to indicate that the latter regulatory inputs to fat cells are probably unchanged in women with Cushing's disease.

The ApEn statistic was used to monitor the minute-to-minute pattern reproducibility of leptin release. This function quantifies non-pulsatile and non-circadian regulation of hormone secretion (46). Leptin ApEn values were similar in patients and controls, which signifies comparable feedback and feedforward control of leptin secretion. In contrast, in neuroendocrine tumoral states, secretion is characterized by increased disorderliness (high ApEn), mirroring loss of coordinate feedback control (47–49). For example, in relation to adrenocorticotropin (ACTH) and cortisol secretion, Cushing's disease is marked by disruption of orderly ACTH secretion, and by erosion of the joint synchrony between ACTH and cortisol release (50). The unexpected stability of joint cortisol–leptin and leptin–GH secretion in Cushing patients points to maintained fat-cell responsiveness, at least to these two opposing metabotropic signals (cortisol to stimulate leptin production and GH to inhibit leptin production).

In obese patients, leptin gene expression is increased in subcutaneous fat (51). Whether a similar mechanism operates in Cushing's patients is not yet known, but it would account for most of the foregoing amplitude-specific mechanisms driving leptin overproduction, while sparing frequency and regularity measures. Likewise, more subcutaneous fat cells in Cushing patients (52) would explicate many of the present new findings, as well as the early observation (above) of delayed resolution of hyperleptinemia after surgical curing of Cushing's syndrome.

References

- 1 Trayhurn P, Hoggard N, Mercer JG & Rayner DV. Leptin: fundamental aspects. *International Journal of Obesity and Related Metabolic Disorders* 1999 **23** Suppl 1 22–28.
- 2 Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H *et al.* The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proceedings of the National Academy of Sciences of the USA* 1996 **93** 8374–8378.
- 3 Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine* 1996 **334** 292–295.
- 4 Hamilton BS, Paglia D, Kwan AY & Deitel M. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nature Medicine* 1995 **1** 953–956.
- 5 Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F & Leibel RL. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3424–3427.
- 6 Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q & Garvey WT. The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin sensitivity, and energy expenditure. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1293–1300.
- 7 Ahmed ML, Ong KK, Morrell DJ, Cox L, Drayer N, Perry L *et al.* Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 899–905.
- 8 De Vos P, Saladin R, Auwerx J & Staels B. Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *Journal of Biological Chemistry* 1995 **270** 15958–15961.
- 9 Murakami T, Iida M & Shima K. Dexamethasone regulates obese expression in isolated rat adipocytes. *Biochemical and Biophysical Research Communications* 1995 **214** 1260–1267.
- 10 Pappaspyrou-Rao S, Schneider SH, Petersen RN & Fried SK. Dexamethasone increases leptin expression in humans *in vivo*. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1635–1637.
- 11 Dagogo-Jack S, Selke G, Melson AK & Newcomer JW. Robust leptin secretory responses to dexamethasone in obese subjects. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3230–3233.
- 12 Leal-Cerro A, Considine RV, Peino R, Venegas E, Astorga R, Casanueva FF & Dieguez C. Serum immunoreactive-leptin levels are increased in patients with Cushing's syndrome. *Hormone and Metabolic Research* 1996 **28** 711–713.
- 13 Cizza G, Litsikas AJ, Licinio J, Gold PW & Chrousos GP. Plasma leptin levels do not change in patients with Cushing's disease shortly after correction of hypercortisolism. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2747–2750.
- 14 Biemond P, de Jong FH & Lamberts SJW. Continuous dexamethasone infusion for seven hours in patients with the Cushing syndrome: a superior differential diagnostic test. *Annals of Internal Medicine* 1990 **112** 738–742.
- 15 Veldhuis JD & Johnson ML. Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. *American Journal of Physiology* 1986 **250** E486–E493.
- 16 Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB *et al.* Human leptin levels are pulsatile and inversely related to pituitary–adrenal function. *Nature Medicine* 1997 **3** 575–579.
- 17 Licinio J, Negrao AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB *et al.* Sex differences in circulating human leptin pulse amplitude: clinical implications. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 4140–4147.
- 18 Veldhuis JD & Johnson ML. Deconvolution analysis of hormone data. *Methods in Enzymology* 1992 **210** 539–575.
- 19 Veldhuis JD, Moorman J & Johnson ML. Deconvolution analysis of neuro-endocrine data: waveform-independent methods and applications. *Methods in Neurosciences* 1994 **20** 279–325.
- 20 Klein S, Coppack SW, Mohamed-Ali V & Landt M. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes* 1996 **45** 984–987.
- 21 Pincus SM. Approximate entropy as a measure of system complexity. *PNAS* 1991 **88** 2297–2301.
- 22 Pincus SM & Singer B. Randomness and degrees of irregularity. *PNAS* 1996 **93** 2083–2088.
- 23 Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP & Ravussin E. Effects of glucocorticoids on energy metabolism and food intake in humans. *American Journal of Physiology* 1996 **271** E317–E325.
- 24 Takahashi M, Funahashi T, Shimomura I, Miyaoka K & Matsuzawa Y. Plasma leptin levels and body fat distribution. *Hormone and Metabolic Research* 1996 **28** 751–752.
- 25 Langendonk JG, Pijl H, Toornvliet AC, Burggraaf J, Frölich M, Schoemaker RC *et al.* Circadian rhythm of plasma leptin levels in upper and lower body obese women: influence of body fat distribution and weight loss. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1706–1712.
- 26 Van Harmelen V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, Lonnqvist F *et al.* Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes* 1998 **47** 913–917.
- 27 Wajchenberg BL, Bosco A, Marone MM, Levin S, Rocha M, Lerario AC *et al.* Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 2791–2794.
- 28 Gill MS, Toogood AA, O'Neill PA, Adams JE, Thorner MO, Shalet SM & Clayton PE. Relationship between growth hormone

- (GH) status, serum leptin and body composition in healthy and GH deficient elderly subjects. *Clinical Endocrinology* 1997 **47** 161–167.
- 29 Nicklas BJ, Toth MJ, Goldberg AP & Poehlman ET. Racial differences in plasma leptin concentrations in obese postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 315–317.
 - 30 Isosaki O, Tsushima T, Miyakawa M, Nozoe Y, Demura H & Seki H. Growth hormone directly inhibits leptin gene expression in visceral fat tissue in fatty Zucker rats. *Journal of Endocrinology* 1999 **161** 511–516.
 - 31 Janssen YJJ, Frölich M, Deurenberg P & Roelfsema F. Serum leptin levels during recombinant human GH therapy in adults with GH deficiency. *European Journal of Endocrinology* 1997 **137** 650–654.
 - 32 Fisker S, Vahl N, Hansen TB, Jorgensen JOL, Hagen C, Orskov H. *et al.* Serum leptin is increased in growth hormone-deficient adults: relationship to body composition and effects of placebo-controlled growth hormone therapy for 1 year. *Metabolism* 1997 **46** 812–817.
 - 33 Veldhuis JD, Iranmanesh A, Ho KK, Waters MJ, Johnson ML & Lizarralde G. Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 51–59.
 - 34 Roubenoff R, Rall LC, Veldhuis JD, Kehayias JJ, Rosen C, Nicolson M. *et al.* The relationship between growth hormone kinetics and sarcopenia in postmenopausal women: the role of fat mass and leptin. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1502–1506.
 - 35 Groote Veldman R, Frölich M, Pincus SM, Veldhuis JD & Roelfsema F. Growth hormone and prolactin are secreted more irregularly in patients with Cushing's disease. *Clinical Endocrinology* 2000 **52** 625–632.
 - 36 Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R. *et al.* Acute and chronic effects of insulin on leptin production in humans: studies *in vivo* and *in vitro*. *Diabetes* 1996 **45** 699–701.
 - 37 Boden G, Chen X, Kolaczynski JW & Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *Journal of Clinical Investigation* 1997 **100** 1107–1113.
 - 38 Carantoni M, Abbasi F, Azhar S, Schaaf P & Reaven GM. Can changes in plasma insulin concentration explain the variability in leptin response to weight loss in obese women with normal glucose tolerance? *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 869–872.
 - 39 Gomez JM, Molina A, Fernandez-Castaner M, Casamitjana R, Martinez-Matos JA & Soler J. Insulin regulation of leptin synthesis and secretion in humans: the model of myotonic dystrophy. *Clinical Endocrinology* 1999 **50** 569–575.
 - 40 Weise M, Abad V, Considine RV, Nieman L & Rother KI. Leptin secretion in Cushing's syndrome: preservation of diurnal rhythm and absent response to corticotropin-releasing hormone. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2075–2079.
 - 41 Widjaja A, Schürmeyer TH, von zur Mühlen A & Brabant G. Determinants of serum leptin levels in Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 600–693.
 - 42 Laughlin GA, Morales AJ & Yen SS. Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance/hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1692–1696.
 - 43 Mantzoros CS, Dunaif A & Flier JS. Leptin concentrations in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1687–1691.
 - 44 Chapman IM, Wittert GA & Norman RJ. Circulating leptin concentrations in polycystic ovary syndrome: relation to anthropometric and metabolic parameters. *Clinical Endocrinology* 1997 **46** 175–181.
 - 45 Simon C, Gronfier C, Schlienger JL & Brandenberger G. Circadian and ultradian variations of leptin in normal man under continuous enteral nutrition: relationship to sleep and body temperature. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1893–1899.
 - 46 Veldhuis JD & Pincus SM. Orderliness of hormone release patterns: a complementary measure to conventional pulsatile and circadian analyses. *European Journal of Endocrinology* 1998 **138** 358–362.
 - 47 Hartman ML, Pincus SM, Johnson ML, Hunter Matthews D, Faunt ML, Vance LM. *et al.* Enhanced basal and disorderly growth hormone secretion distinguish acromegalic from normal pulsatile growth hormone release. *Journal of Clinical Investigation* 1994 **94** 1277–1288.
 - 48 Groote Veldman R, van den Berg G, Frölich M, Pincus SM, Veldhuis JD & Roelfsema F. Increased episodic release and disorderliness of prolactin secretion in both micro- and macro-prolactinomas. *European Journal of Endocrinology* 1990 **140** 192–200.
 - 49 Van den Berg G, Pincus SM, Veldhuis JD, Frölich M & Roelfsema F. Greater disorderliness of ACTH and cortisol release accompanies pituitary-dependent Cushing's disease. *European Journal of Endocrinology* 1997 **136** 394–400.
 - 50 Roelfsema F, Pincus SM & Veldhuis JD. Patients with Cushing's disease secrete adrenocorticotropin and cortisol jointly more asynchronously than healthy subjects. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 688–692.
 - 51 Van Gaal LF, Wauters MA, Mertens IL, Considine RV & de Leeuw IH. Clinical endocrinology of human leptin. *International Journal of Obesity and Related Metabolic Disorders Suppl 1* 1999 29–36.
 - 52 Krotkiewski M, Blohme B, Lindholm N, Bjorntorp P. The effect of adrenal corticosteroids on regional adipocyte size in man. *Journal of Clinical Endocrinology and Metabolism* 1976 **42** 91–97.

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