Human and clinical perspectives on leptin

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Body-weight regulation has for a long time been considered to be mainly a question of 'to eat or not to eat', subject to free will. However, a simple calculation sheds doubt on this view; a normal weight person of 60 years has ingested about 230×10^6 kJ during his life and, based on thermodynamics, has consumed exactly the same amount of energy, disregarding the amount of chemical energy stored during body growth in early life. If the daily energy intake had exceeded the energy expenditure by only 2 %, which is not more than a good mouthful of milk, this person would weigh about 200 kg today. If the daily energy intake had been 1 % less than the expenditure, this person would have starved to death many years ago. This simple consideration suggests that body-weight regulation must be subject to mechanisms which balance the energy influx and efflux in an extremely fine-tuned manner.

The search for such mechanisms started about 40 years ago in the 'golden era of physiology', using parabiosis experiments in rodents. These led to the conclusion that a humoral factor must exist which is produced by adipose tissue and which is pivotal for the regulation of body weight (Kennedy, 1953; Hausberger, 1958; Hervey, 1959; Coleman & Hummel, 1969). However, the most straightforward approach to the isolation and characterization of this factor by biochemical techniques failed. Solving this problem could be achieved only recently using modern techniques of molecular genetics, when J. Friedman and his collaborators (Zhang *et al.* 1994) cloned the gene from *ob/ob* mice. Subsequently, the gene was cloned and expressed by a number of groups, and the gene product, which was termed 'leptin', referring to the Greek word leptós meaning thin (Halaas *et al.* 1995), became available for animal and human studies. During this short time, the knowledge regarding this new hormone 'exploded' in the real sense of the word. From animal experiments it became evident that leptin is not only pivotal for the regulation of body weight, but that it is also an integral component of various endocrine axes or, more correctly, of various endocrine feedback loops (Ahima *et al.* 1996; Barash *et al.* 1996; Chehab *et al.* 1996, 1997). Teleologically, this makes sense; the information on available energy stores provided by leptin is extremely important for the organism with respect to many key functions, such as maintenance of body weight, reproduction or growth.

The availability of recombinant human leptin (Lilly Research Laboratories) enabled us to develop a highly-sensitive, accurate and robust radioimmunoassay to study serum leptin in human subjects (Blum *et al.* 1997*a*). Most importantly, the high sensitivity of the assay also allowed us to measure leptin precisely and accurately in patients and healthy individuals with low levels, such as very lean patients, young children or adolescent males. The current project to measure leptin levels in healthy subjects and in patients with diverse disorders had the following aims:
to define major controlling variables in human subjects;
to construct reference ranges with respect to these major variables;
to investigate the regulation of circulating leptin in comparison with animal and *in vitro* experiments;
to search for pathological situations in which abnormal leptin production may possibly play a causal role.

Since studies in human subjects rarely allow well-controlled manipulations to the same extent as in animal experiments and, in contrast to *in vitro* experiments, always consider a most complex *in vivo* situation where many variables may interact, their potency to prove cause–effect relationships is limited. Any associations found must be examined, therefore, in terms of whether they are consistent with hypotheses derived from animal studies and *in vitro* experiments. This dual and complementary approach may then lead to theories on how leptin is regulated and how it acts in human subjects. On this basis, any role for leptin in the pathophysiology of disease states, such as obesity, eating disorders or infertility, may finally be derived.

Constitutive variables controlling serum leptin: fat mass and androgens

When we studied serum leptin levels in large cohorts of healthy children, adolescents and adults (about 2000 individuals), it became evident that body fat is the major determinant of circulating leptin. This observation has also been made by many other groups (Considine *et al.* 1996; Horn *et al.* 1996; Blum *et al.* 1997*a*) and is consistent with findings in rodents (Frederich *et al.* 1995; Maffei *et al.* 1995; Considine *et al.* 1996). It is not surprising that adipose tissue is the main source of leptin. The relationship between leptin levels

Abbreviations: GH, growth hormone; IGF-1, insulin-like growth factor-1.

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and measures of body fat mass, such as BMI and percentage body fat (determined by bioelectric impedance measurements or by dual energy X-ray absorptiometry), revealed an exponential characteristic, indicating that small changes in body fat result in relatively large changes in serum leptin (Blum *et al.* 1997*a*) and explaining the tremendous variation in leptin levels of over three orders of magnitude.

During sexual maturation, a divergent pattern has been observed in both sexes (Fig. 1). While in girls leptin levels increased steadily with pubertal development, the levels were highest in boys during early puberty (Tanner stage 2; Tanner & Whitehouse, 1976) and declined thereafter, resulting in about 8-fold higher leptin levels in females compared with males at Tanner stage 5, although BMI values were comparable (Blum *et al.* 1997*a*; Clayton *et al.* 1997). From animal studies in rodents it has become clear that leptin plays a key role in allowing puberty to proceed (Ahima *et al.* 1997; Chehab *et al.* 1997; Cheung *et al.* 1997; Pierroz *et al.* 1997). It may be hypothesized, therefore, that leptin has a permissive role in relation to pubertal development in human subjects also, although it is hard to conceive that leptin *per se* triggers puberty (Mantzoros *et al.* 1997). This finding provides an explanation for the hypothesis of Frisch (1980), known to paediatricians for a long time, that a critical fat mass is required for puberty to occur.

A sex-specific pattern was also established, with leptin levels being related to BMI. While the exponential regression lines for leptin *v.* BMI in both sexes were almost identical at Tanner stages 1 and 2, there was a marked decline in boys at a given BMI value during further progression of puberty. This decline was much less pronounced in girls (Blum *et al.* 1997*a*). To exclude the possibility that the divergent developmental pattern of body composition, with males increasing their BMI during puberty mainly through an increase of muscle mass and girls mainly through accumulation of fat mass, is the cause of this phenomenon, leptin levels were also related to body fat determined by bioelectric impedance measurements. However, a significant sex difference remained (Blum *et al.* 1997*a*), suggesting the presence of a sex-specific regulator other than fat mass.

In large cohorts of healthy adults of both sexes (more than 1200) between 20 and 80 years of age, a clear sex difference was obvious, with higher levels in females even after adjustment for BMI, in accordance with reports by others (Hickey *et al.* 1996; Horn *et al.* 1996; Ma *et al.* 1996; Saad *et al.* 1997). However, in adults, age did not significantly contribute to the variation in leptin levels, as shown by multiple-regression analysis.

The question remains as to which factors cause the sex difference in serum leptin concentrations. The fact that this difference becomes evident at the time when sex steroids rise during puberty indicated that either stimulation by oestrogens or suppression by androgens may be involved. From various studies, including *in vitro* experiments with human adipocytes, there was no evidence that oestrogens would play a role. However, a significant inverse correlation between leptin levels and serum testosterone was found in healthy boys and male adolescents (Fig. 2). In contrast to many other endocrine factors, this relationship remained significant when applying multiple-regression analysis irrespective of the models used (Blum *et al.* 1997*a*). Similar findings were also obtained in obese children and adolescents (Wabitsch *et al.* 1997) and in obese adults (Vettor *et al.* 1997), suggesting that androgens, especially testosterone, suppress leptin production. This evidence was reinforced by more conclusive studies in hypogonadal men and in trans-sexual subjects. In hypogonadal men, leptin levels adjusted for BMI were elevated when compared with those for healthy men, and normalized on testosterone substitution. When testosterone levels declined, serum leptin increased again to pretreatment values (Jockenhövel *et al.* 1997). These changes occurred within a couple of weeks. In trans-sexuals, leptin levels reversed according to the sex steroid-induced change in the phenotype (Elbers *et al.* 1997). The most conclusive evidence, however, stems from *in vitro* experiments with human adipocytes, in which leptin production was directly suppressed by testosterone in a dose-dependent manner (Wabitsch *et al.* 1997).

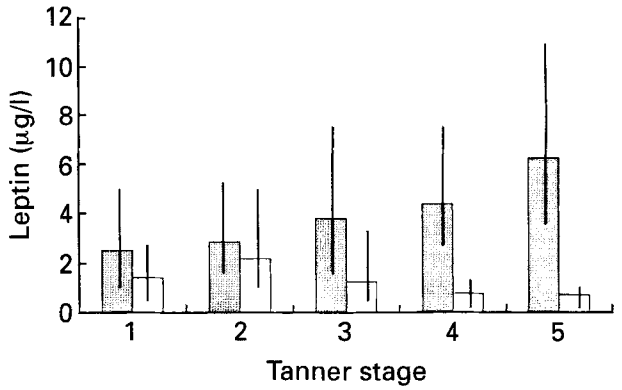

Fig. 1. Serum leptin levels in girls (▨) and boys (□) at various pubertal stages. Values are medians and ranges represented by vertical bars; ranges were derived from +1 SD to −1 SD after logarithmic transformation of the leptin values.

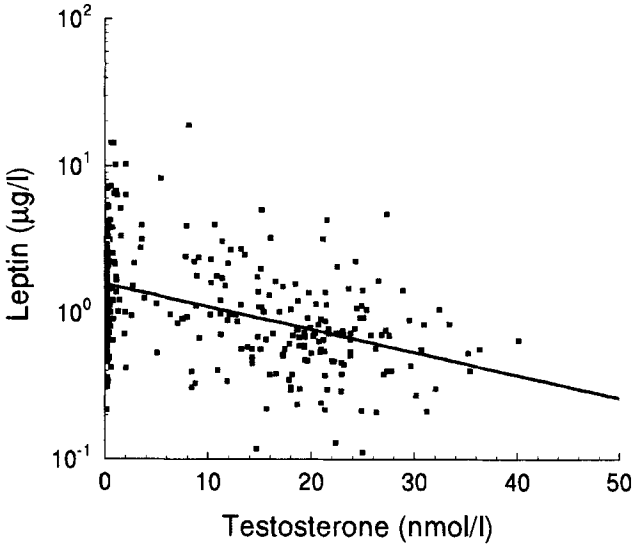

Fig. 2. Serum leptin levels in boys and male adolescents between 5 and 20 years of age *v.* testosterone levels. *n* 310, *r* −0·43, $P < 0{\cdot}0001$.

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In summary, the major regulators of serum leptin levels are body fat mass and androgen levels. Together, they determine the constitutive synthesis of leptin. Any alterations in serum leptin due to changes in either of these variables follow a slow pattern, in contrast to the relatively rapid regulation superimposed by a number of other factors (discussed later).

Construction of reference ranges

In order to decide whether a serum leptin concentration for a given patient is as expected or whether it is abnormal, reference ranges are required. These should be referred to the variable that predominantly influences leptin levels, body fat mass, and should be stratified according to sex and stage of sexual maturation. For practical reasons, the independent variable should be easily accessible. A readily-available measure of body fat is the BMI, although it is certainly not very accurate. In contrast to other more sophisticated methods, however, it provides a number of advantages: (1) it can be easily determined by everyone everywhere; (2) it does not require expensive and technically-demanding equipment; (3) it is generally retrospectively available; (4) it reflects longitudinal short-term changes in body fat quite precisely; (5) it is already widely used for defining overweight and obesity, and thus provides an accepted frame for the classification of patients.

For these reasons, leptin reference ranges were based on BMI and were stratified according to sex and stages of sexual maturation. Equations were derived which define the 90 % inclusion limits of normal leptin levels (Table 1) and which allow the calculation of standard deviation scores (Z-scores) adjusting serum leptin concentrations for BMI, sex and developmental stage (Blum *et al.* 1997*a*; Blum & Juul, 1997).

Of course, in a strict sense, these ranges are limited to the BMI ranges of the investigated cohorts. At very low or very high BMI values the relationship with leptin may deviate from the one obtained by simple mathematical extrapolation. Thus, the ranges given in Table 1 should rather be considered as 'expectation ranges'. Nevertheless, these simple mathematical tools have proved to be very useful for detection of abnormal leptin production, and revealed influences of regulators other than the major confounding variables (Table 2).

In summary, reference ranges for serum leptin levels should be referred to body fat mass, e.g. the BMI, and should be stratified according to sex and stage of sexual maturation.

Short-term regulation of serum leptin

Diurnal variation

Leptin levels exhibited a circadian variation in various groups of healthy individuals or patients with a peak at about 02.00 hours. This nocturnal peak exceeds daytime values by about 30–100 % and is present in both rodents and human subjects (Sinha *et al.* 1996), although the rat is a night feeder and man normally eats during the day. At first sight, therefore, this increase does not appear to be related to food intake. However, during fasting the nocturnal peak was absent. Taking into account the fact that the response of leptin to insulin is much faster in rodents than in human subjects (Pagano *et al.* 1997), it may be concluded that the rise in leptin during the night may be induced by insulin, with a short time lag in rodents and a longer lag phase in human subjects. By cross-correlation analysis we have shown that the maximum mean insulin concentration precedes the leptin peak by 6 h in human subjects.

The diurnal variation in serum leptin levels suggests that serial blood sampling is required for the proper assessment of the leptin secretory status. This may, in fact, be true in specific situations (Blum *et al.* 1997*b*). For most applications, however, a single measurement in the morning or early afternoon is sufficient and informative.

Food intake

In a group of healthy adults (n 10), fasting for 3 d induced rapid and pronounced changes in leptin levels. After 24 h of complete food restriction, leptin fell to about 50 % of basal levels and after 33 h to about 30 % of the basal values, and remained stable on continuous fasting (Blum *et al.* 1997*b*). This finding compares well with reports by others in human subjects and rodents (Kolaczynski *et al.* 1996*a*; Pierroz *et al.* 1997). Resumption of normal eating cycles caused a rapid rise in leptin levels to almost basal values after 9 h. Insulin

Table 1. Reference ranges of serum leptin levels referring to BMI stratified according to sex and developmental stage

(The best-fit regression lines for the various subgroups are exponential curves of the form leptin = $a^{*}e^{b^{*}BMI}$. For sufficiently large numbers this curve corresponds to the 50th percentile. The 5th and 95th percentiles are given by the following equations: leptin = $a^{*}e^{b^{*}BMI-c}$ and leptin = $e^{b^{*}BMI+c}$ respectively. In a semi-logarithmic plot (y-axis = log leptin), these curves give straight lines. Accounting for the logarithmic distribution of leptin levels, the leptin standard deviation scores (SDS; Z-score) can be calculated by the following equation: leptin SDS = (ln(leptin) – ln(a) – b*BMI)/d)

Cohort	n	a	b	c	d
Males:					
TS 1 and 2	136	0·0146	0·2706	0·8821	0·5379
TS 3 and 4	50	0·0181	0·2067	1·1919	0·6850
TS 5	112	0·0316	0·1462	1·0821	0·6558
Adults	380	0·0130	0·2200	1·1053	0·6740
Females:					
TS 1 and 2	136	0·0422	0·2499	0·7849	0·4786
TS 3 and 4	43	0·0543	0·2357	0·5745	0·3379
TS 5	157	0·2550	0·1508	0·7053	0·4301
Adults	587	0·3042	0·1467	0·8548	0·5212

TS, Tanner stage; ln, natural logarithm.

Table 2. Variables that control serum leptin levels

Stimulating	Suppressing
Body fat mass	Androgens
Overfeeding	Fasting
Impaired renal function	Sympathicotonus
Insulin	Growth hormone
Glucocorticoids	Catecholamines
Tumour necrosis factor	Cold exposure
Interleukin-1	Physical exercise

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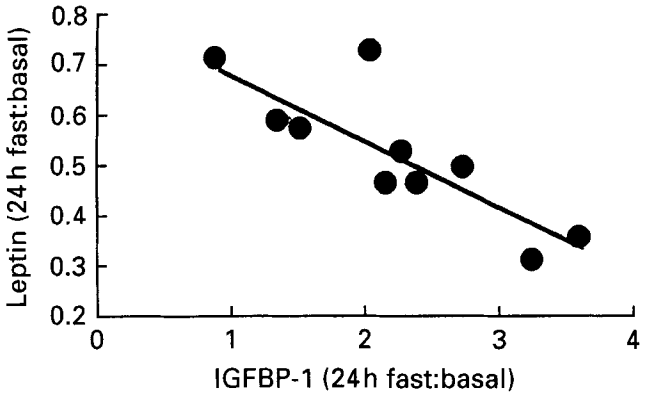

Fig. 3. Decrease in serum leptin *v.* the increase of insulin-like growth factor-binding protein (IGFBP)-1 after a 24 h fast in ten healthy young adults. r –0·88, $P < 0·0001$. The *y*-axis represents serum leptin after fasting : non-fasted baseline value and the *x*-axis represents the corresponding ratio for IGFBP-1.

levels followed exactly the same pattern, although correlations between changes in leptin and insulin did not reach statistical significance. However, a very high inverse correlation (r –0·88) was obtained between changes of leptin and insulin-like growth factor (IGF)- binding protein-1 (Fig. 3). The predominant regulator of IGF-binding protein-1 is insulin, which has a suppressive effect. IGF-binding protein-1 is considered a surrogate marker for the activity of insulin in the liver integrated over time. It may be concluded, therefore, that insulin is the link between leptin and its regulation by food intake. In contrast to food restriction, overfeeding was shown to stimulate serum leptin levels (Harris *et al.* 1996; Kolaczynski *et al.* 1996*c*).

Insulin

Early studies in rodents showed that insulin stimulates leptin expression (Saladin *et al.* 1995). This effect became evident after only 2 h, whereas in human subjects no changes were found within this short period (Pagano *et al.* 1997). This was taken as evidence at this early phase of leptin research that insulin is not involved in leptin regulation in man. Since then, however, evidence has accumulated that insulin is also an important regulator in human subjects (Kolaczynski *et al* 1996*b*; Malström *et al.* 1996).

In children with untreated insulin-dependent diabetes mellitus, we found significantly decreased serum leptin levels, adjusted for BMI, sex and pubertal stage (Kiess *et al.* 1998). On insulin therapy, levels normalized and were even elevated post puberty, which may indicate either abnormal body composition with a relative abundance of fat mass, or oversubstitution due to the intensified insulin regimen. Moreover, in a large population-based study we have shown that insulin contributed to the variation in leptin levels independently of BMI. This finding was confirmed by glucose clamp studies, which also showed that the time course of the insulin effect in human subjects is much slower than that in rodents, with no detectable increase before about 6 h (Malström *et al.* 1996). *In vitro* experiments with primary differentiated human adipocytes finally proved that insulin stimulates leptin expression and synthesis in a dose-dependent manner (Wabitsch *et al.* 1996).

Glucocorticoids

Dexamethasone administration in young healthy adults at high doses (8 mg) caused a 3-fold rise in leptin levels within 9 h (Miell *et al.* 1996). A similar stimulating effect was also obtained in obese children and adults (Kiess *et al.* 1996) with a low dose (1 mg), although it was less pronounced. These findings compare well with those from animal studies (De-Vos *et al.* 1995) and *in vitro* experiments, where cortisol showed a synergistic stimulatory effect with insulin (Wabitsch *et al.* 1996). The fact that glucocorticoids influence leptin expression and serum leptin levels is most stringently suggested by the presence of a glucocorticoid response element in the leptin promoter region (Gong *et al.* 1996).

Growth hormone and insulin-like growth factor-1

In a large cohort of adult patients with growth hormone (GH) deficiency, leptin levels did not significantly deviate from the reference range when adjusted for BMI and sex. Surprisingly, however, correlations with various measures of body fat were abnormal in the sense that they were much less than those for cohorts of healthy individuals. They improved substantially on GH substitution (Attanasio *et al.* 1996), suggesting that GH is involved somehow, either directly or indirectly, in the regulation of leptin. In adolescents and young adults with GH deficiency, GH treatment caused a substantial increase in the nocturnal leptin peak during the second night after commencing substitution. Whether this effect was due to an increase in insulin or direct GH effects remains open. After 4 weeks of GH replacement, this increase had disappeared (Blum *et al.* 1997*c*). In children with isolated GH deficiency, BMI-, sex- and pubertal stage-adjusted leptin levels were normal before therapy and decreased by 30 % after 4 weeks of GH therapy, which could not be explained by changes in body composition (Rauch *et al.* 1997). This finding is consistent with the suppression of leptin production to a similar degree in human adipocytes *in vitro* (M. Wabitsch, personal communication).

In patients with GH-receptor defect (Laron syndrome), IGF-1 treatment did not cause significant changes in leptin levels (Laron *et al.* 1997). This is consistent with *in vitro* findings in human adipocytes, in which IGF-1 stimulated leptin expression only at unphysiologically high concentrations, probably through the insulin receptor.

In summary, the influence of GH and IGF-1 on leptin levels is less clear. From our own studies we must conclude that GH exerts a short-term stimulatory effect and a long-term suppressive effect, consistent with *in vitro* findings. IGF-1 does not appear to influence leptin expression, in accordance with the absence of effective receptors on adipocytes.

Thyroid hormones

In large cohorts of patients with hypo- or hyperthyroidism we found no abnormal leptin levels adjusted for BMI

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and sex. Furthermore, adequate treatment correcting the disease state did not cause significant changes in leptin (Corbetta *et al.* 1997), although patients with a leptin-receptor defect were recently shown to have diminished thyrotropin secretion (Clément *et al.* 1998).

Other regulating variables

Animal and *in vitro* studies suggest that a number of additional variables are involved in the regulation of leptin. For example, β_3-adrenoceptor agonists, and other hormonal signals increasing cAMP in adipocytes, have a suppressive effect (Moinat *et al.* 1995; Slieker *et al.* 1996; Donahoo *et al.* 1997) which occurs through cAMP response elements on the promoter region of the *ob* gene (Gong *et al.* 1996). Cytokines such as tumour necrosis factor or interleukin-1 increased leptin in animal studies and also in human subjects, which may explain the decreased food intake in sepsis (Grunfeld *et al.* 1996; Janik *et al.* 1997; Zumbach *et al.* 1997).

Leptin: an integral component of metabolic and endocrine feedback loops

Originally, leptin was regarded purely as a satiety signal, but this view has eventually turned out to be too narrow. A major site of leptin action is the hypothalamus, where it suppresses neuropeptide Y expression and activity (Stephens *et al.* 1995; Schwartz *et al.* 1996; Campfield & Smith, 1998). Neuropeptide Y is known to be a major stimulator of appetite, and its suppression may be a main mechanism by which leptin also suppresses food intake, although a number of other mediators may also be involved. From the hypothalamus, signals are transferred to the central nervous system, which finally stimulate energy expenditure.

Interestingly, apart from its effects on energy influx and efflux, leptin was shown also to exert major effects on the various hypothalamo–pituitary–endocrine axes in rodents. Stimulation of GH and gonadotropins and suppression of adrenocorticotrophic hormone was reported (Ahima *et al.* 1996; Barash *et al.* 1996; Carro *et al.* 1997; Chehab *et al.* 1997; Vuagnat *et al.* 1998). Again, these effects may well be explained by suppression of neuropeptide Y. It should be mentioned that corresponding findings in human subjects are still unavailable at present. On the other hand, taking the regulatory influences on leptin levels of GH, testosterone and glucocorticoids into account, these findings call for reconsideration of the various hypothalamo–pituitary–endocrine feedback loops. It appears that they should be extended by two components, neuropeptide Y on one side and leptin on the other side (Fig. 4). In a simplistic view, these regulatory loops represent classical negative feedback loops which aim principally at the stabilization of a given equilibrium.

Leptin in weight disorders

Since leptin's role is that of an indicator of body fat mass, a major goal of leptin research is to delineate its importance in the pathophysiology of abnormal weight regulation such as obesity or anorexia. In the end, these efforts may help to reveal new disease entities which may be causally related to the synthesis or action of leptin.

Obesity

Most obese patients, both adults and children, have increased leptin levels (Considine *et al.* 1996; Ma *et al.* 1996; Wabitsch *et al.* 1997) which fall within the reference range when adjusted for sex, developmental stage and BMI (Fig. 5). This finding suggests the presence of some kind of leptin insensitivity. A number of possible mechanisms causing leptin insensitivity may be conceived, such as limited transportation capacity across the blood–brain barrier (Caro *et al.* 1996), a leptin-receptor defect (Clément *et al.* 1998), impaired post-receptor signal transduction, or even defective mechanisms further down-stream of the cascade of interactions which are involved in the regulation of balancing energy influx and efflux. However, in a small percentage of patients leptin levels were clearly lower than one would expect from their fat mass. In fact, these are mostly the patients with excessively high body weight. It may be speculated, for example, that stimulation by certain regulators such as insulin is impaired, or that leptin synthesis in adipocytes is defective. Extremely obese patients with a mutation of the leptin gene causing virtually complete absence of leptin, analogous to the *ob/ob* mouse, have been described (Montague *et al.* 1997).

During dietary programmes for weight reduction, leptin levels adjusted for sex and BMI decreased further within the first couple of weeks than one would have expected from weight loss, suggesting a relative leptin deficiency in this phase. This may explain some of the problems encountered with weight-reduction programmes (Scholz *et al.* 1996).

In summary, it appears that at least two forms of 'normal' obesity exist, one with absolute or relative leptin deficiency and another more frequent one with relative leptin insensitivity. By analogy to diabetes mellitus, they may be classified as type 1 and type 2 obesity. Future research defining the

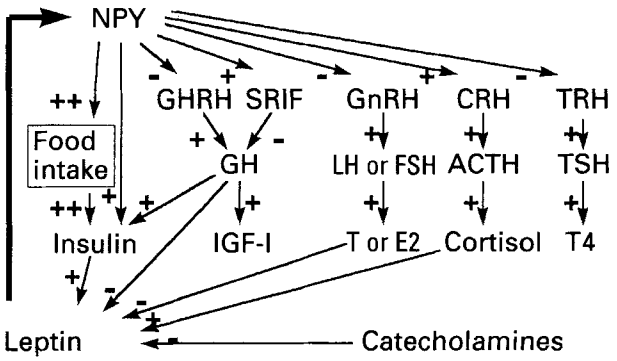

Fig. 4. Leptin and neuropeptide Y (NPY) as integral components of metabolic and endocrine feedback loops. +, Stimulation; –, suppression; GHRH, growth hormone-releasing hormone; SRIF, somatostatin; GH, growth hormone; IGF-1, insulin-like growth factor-1; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testosterone; E2, 17β-oestradiol; CRH, corticotrophin-releasing hormone; ACTH, adrenocorticotrophic hormone; TRH, thyrotrophin-releasing hormone; TSH, thyroid-stimulating hormone; T4, thyroxine.

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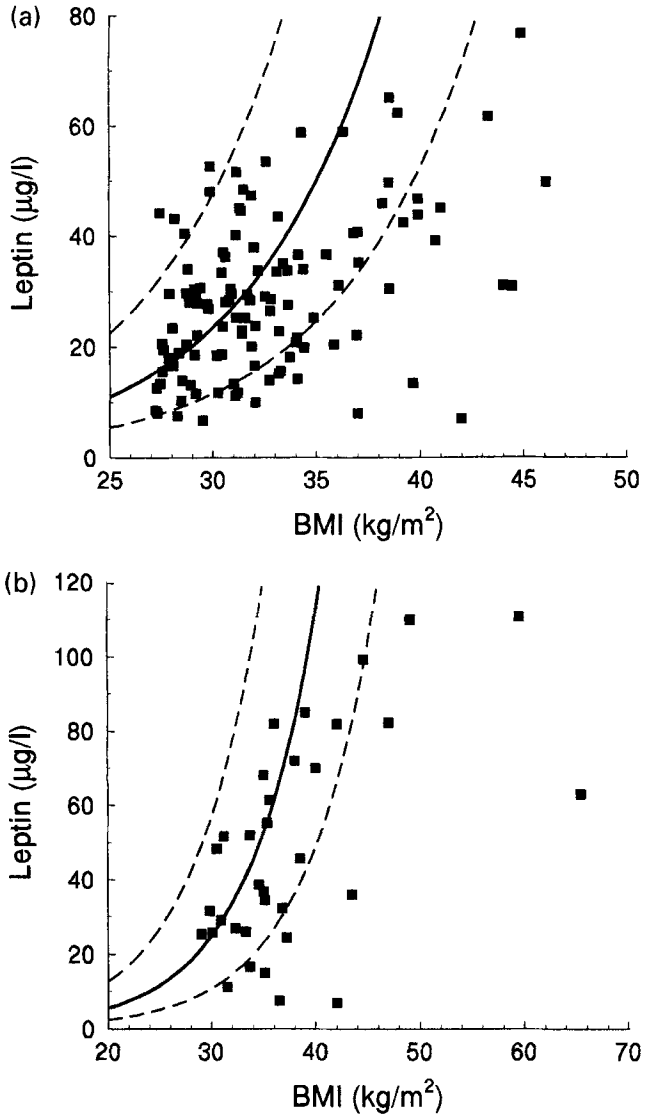

Fig. 5. Leptin levels *v.* BMI in (a) obese girls and female adolescents and (b) in obese women older than 20 years. The expectation ranges are shown for the 5th and 95th percentiles (- - -) and the 50th percentile (—).

specific molecular defects will show whether this rough classification can be refined.

We have also investigated leptin levels in a number of patients with obesity syndromes. In children with Prader–Labhardt–Willi syndrome (*n* 20), serum leptin was found to be elevated by 1·4 SD on average when adjusted for sex, pubertal stage and BMI. These patients suffer from a hypothalamic defect which causes abnormal eating behaviour, hypogonadism and at least partial GH deficiency. The relatively increased leptin levels may either be explained by an abnormal body composition with a relative abundance of body fat due to insufficient GH and gonadotropin secretion, or by the absence of a putative hypothalamic factor which exerts a negative feedback on leptin synthesis. Theoretically, this absent putative factor could be androgens due to hypogonadism. However, because most of the patients were pre-pubertal, this possibility is unlikely. Also, insulin does not seem to play a role, because insulin levels were decreased (Eiholzer *et al.* 1997). Overall, it appears that leptin synthesis is appropriate, but these patients have a leptin resistance due to their hypothalamic defect.

In children with Laurence–Moon–Bardet–Biedl syndrome (*n* 5), adjusted leptin levels were in the 'expectation range' suggesting that leptin synthesis is normal. In patients with a hypothalamic defect due to radiation therapy (*n* 31), leptin levels were elevated compared with matched controls. This finding is particularly interesting, because it suggests the existence of a (defective) negative feedback loop from the hypothalamus back to the adipose tissue. The nature of this feedback loop is unclear at present, although it may be speculated that impaired GH secretion or a reduced sympathetic tone may be involved.

Anorexia nervosa

In anorectic patients leptin levels were below the reference range for age, BMI and percentage of body fat (Hebebrand *et al.* 1997). This finding may explain some of the characteristic symptoms of this disorder, such as reduced energy expenditure and amenorrhoea. In a cohort of females with either anorexia nervosa, bulimia or restraint eating we could quite precisely define a threshold of serum leptin concentration of 1·85 μg/l, below which irregularities of menstrual cycles occurred (Köpp *et al.* 1997). After hospital admission and during weight gain, leptin levels started to rise rapidly when reaching a BMI threshold of about 16 kg/m² and clearly exceeded the normal range (Hebebrand *et al.* 1997). It may be hypothesized that this inappropriate increase causes suppression of appetite and stimulation of energy expenditure, and may thus explain the difficulty in further increasing weight (Hebebrand *et al.* 1997).

Chronic renal failure

A characteristic feature of children with chronic renal failure is reduced appetite. To investigate whether leptin is involved in the pathophysiology of this phenomenon, a large cohort of patients with chronic renal failure (*n* 124), including twenty-six patients with end-stage renal failure, was studied. A significant inverse correlation between serum leptin levels, adjusted for sex, pubertal stage and BMI, and the glomerular filtration rate was observed, suggesting that the kidneys are involved in the clearance of serum leptin. Furthermore, an inverse relationship was also obtained for adjusted leptin levels *v.* daily energy intake. It may be hypothesized, therefore, that impaired renal function causes an inappropriate increase in circulating leptin, which in turn suppresses appetite (Daschner *et al.* 1998).

Conclusions

The major constitutive determinants of serum leptin levels in human subjects are body fat mass and androgens. Suppression by testosterone is the most likely explanation for the well-established sex difference, with lower levels in men. Reference ranges therefore, should refer to body fat mass and should be stratified according to sex and pubertal stage. Changes in circulating leptin following variations in these

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constitutive determinants are slow and occur within several weeks. This constitutive regulation of leptin is significantly modulated by short-term regulators such as insulin or glucocorticoids; short-term in this context means several hours to 1 d. These relatively rapid changes should be taken into account when interpreting leptin measurements in single samples.

So far, findings from clinical studies with respect to leptin regulation are consistent with animal and *in vitro* experiments, except that leptin variations in rodents seem to be more pronounced and more rapid. Conclusions from these experimental approaches appear, therefore, to have a high explanatory value in relation to the human situation.

In most obese patients, including those with various obesity syndromes, leptin levels are high, although they fall within the expected range according to the patient's body fat mass. In a small percentage, however, leptin levels are clearly lower than expected, suggesting the existence of partial (or in the rare extreme case, complete) leptin deficiency, which may be causally involved in the pathogenesis of fat accumulation and, potentially, endocrine derangements. Obesity, therefore, should no longer be considered a disorder *per se* but a symptom of distinct underlying disease entities. Clinical investigations of serum leptin may help to eventually unravel the pathophysiology of some obesity disorders. There is no doubt, however, that body-weight regulation is most complex, and that many other defects apart from abnormal leptin synthesis will be discovered in the future. Certainly, obesity research is no longer at the stage when body weight was considered only a question of 'to eat or not to eat'.

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