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Neuroendocrine Regulation and Actions of Leptin

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The discovery of the adipocyte-produced hormone leptin has greatly changed the field of obesity research and our understanding of energy homeostasis. It is now accepted that leptin is the afferent loop informing the hypothalamus about the state of fat stores, with hypothalamic efferents regulating appetite and energy expenditure. In addition, leptin has a role as a metabolic adaptator in overweight and fasting states. New and previously unsuspected neuroendocrine roles have emerged for leptin. In reproduction, leptin is implicated in fertility regulation, and it is a permissive factor for puberty. Relevant gender-based differences in leptin levels exist, with higher levels in women at birth, which persist throughout life. In adult life, there is experimental evidence that leptin is a permissive factor for the ovarian cycle, with a regulatory role exerted at the hypothalamic, pituitary, and gonadal levels, and with unexplained changes in pregnancy and postpartum. Leptin is present in human milk and may play a role in the adaptive responses of the newborn. Leptin plays a role in the neuroendocrine control of GH secretion, through a complex interaction at hypothalamic levels with GHRH and somatostatin. Leptin participates in the expression of CRH in the hypothalamus, interacts at the adrenal level with ACTH, and is regulated by glucocorticoids. Since leptin and cortisol show an inverse circadian rhythm, it has been suggested that a regulatory feedback is present. Finally, regulatory actions on TRH–TSH and PRL secretion have been found. Thus leptin reports the state of fat stores to the hypothalamus and other neuroendocrine areas, and the neuroendocrine systems adapt their function to the current status of energy homeostasis and fat stores. KEY WORDS: leptin; obesity; anorexia nervosa; growth hormone; puberty; ovarian cycle adrenal function; thyroid axis; prolactin. © 1999 Academic Press

HISTORICAL BACKGROUND

Despite the worldwide medical problem of obesity, one of the most remarkable findings in human nutrition is the stability of body weight. In the industrialized world, the positive energy balance generated by the low physical work needed to obtain food plus the abundance of cheap highly energetic nutrients is minimally reflected by the progressive increase of individual body weight. For example, a normal woman may gain 11 Kg between the ages of 25 and 65, after a total food intake of 20,000 Kg of food in the same period (106). This suggests that the amount of body fat is a factor that is tightly regulated by

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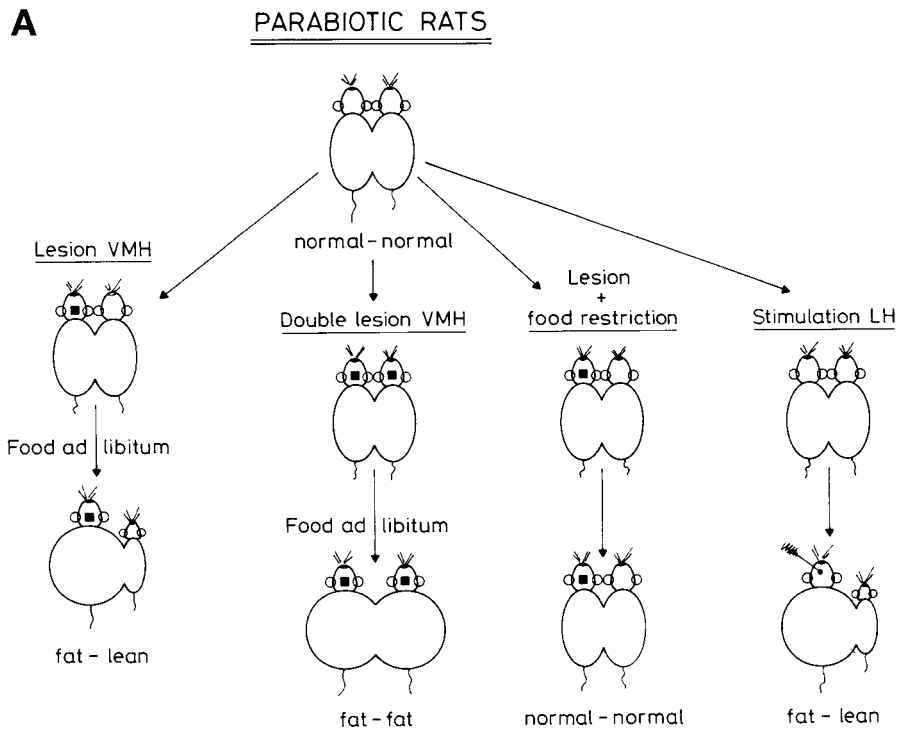
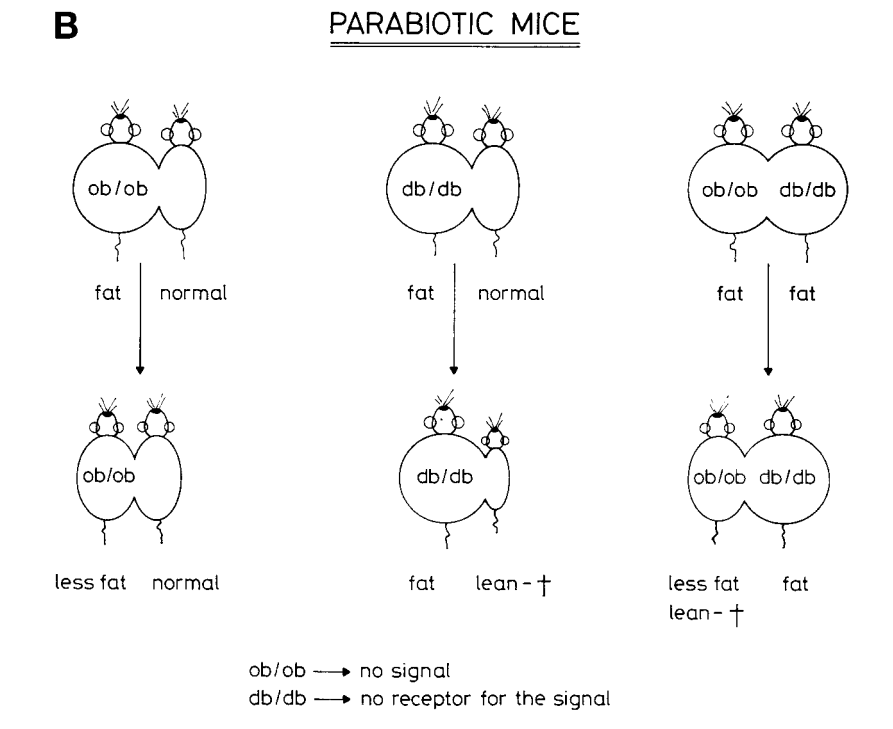
a feedback loop which maintains constancy of total body energy stores (79, 107, 247). To explain this stability, in 1953 Kennedy proposed the "lipostatic theory," in which total body weight is maintained by regulating body fat content (121). He hypothesized that the hypothalamus sensed the concentration of an unknown circulating factor that provided information on the amount of body fat stores. The hypothalamus would then modulate the information to affect changes in food intake to compensate for changes in body fat content (100). In 1959 Hervey addressed the problem using the parabiotic rat model. Parabiosis is a chronic union of a pair of rats by suturing muscles and peritoneum, allowing capillary interconnections but not direct connections of the large vessels. The parabionts are live joined, allowing a permanent exchange of vascular fluids, although this exchange is so slow that only molecules with a long half-life cross from one partner to the other (114). Hervey made one partner of the parabiotic pair hyperphagic by a lesion in the ventromedial hypothalamus, and with the progressive obesity of the lesioned animal, the parabiotic partner become aphagic and lean (105) (Fig. 1A). These results were taken to indicate that an increase in fat mass produced a blood-borne factor which, after crossing to the intact partner, acted to induce satiety. Its lack of effect in the lesioned animal suggested that the receptor for this satiety factor was located at hypothalamic level. These results and interpretations were subsequently confirmed by parabiosis experiments in which rats were made obese by stimulation of the lateral hypothalamus or by gastric tube feeding.

More convincing evidence for a circulating factor in the control of food intake was provided by parabiosis experiments with genetically obese mice (44, 46). Recessive mutations in the mouse obese (*ob*) and diabetes (*db*) genes result in obesity and diabetes in a syndrome resembling morbid human obesity, and *ob*/*ob* and *db*/*db* mice have an identical phenotype, weighing 300% more than normal mice. When a *db*/*db* mouse was parabiosed with a normal one, the *db*/*db* was unaffected but the normal mouse lost weight and died of starvation. When an obese *ob*/*ob* mouse was parabiosed with a normal one, it ate slightly less and the normal mouse was unaffected (Fig. 1B). These results were interpreted as evidence that the *ob*/*ob* mouse did not produce the satiety factor, whereas the *db*/*db* mouse did, but was insensitive to its action due to a defective satiety center (44, 46). This interpretation was confirmed when an *ob*/*ob* and a *db*/*db* were parabiosed: while the *db*/*db* was unaffected the *ob*/*ob*

FIG. 1. (A) Summary of experiments in parabiotic rats (105, 121). When one of the parabionts undergoes a lesion in the ventro medial hypothalamus (VMH) it develops hyperphagia and obesity. This is followed by a progressive loss of body mass in the nonlesioned partner, a change that may be prevented either by avoiding the obesity of the lesioned rat by food restriction or by inducing similar lesion in both rats. Similar results may be obtained by inducing obesity by stimulation of the lateral hypothalamus (LH) or by tube feeding. (B) Summary of experiments in parabiotic mice (44, 46). The parabiosis of an *ob*/*ob* fat mouse with a normal one reduces the obesity of the fat mouse and does not alter the normal mouse. On the other hand, the connection between a *db*/*db* fat mouse and a normal mouse leads to progressive loss of weight of the normal mouse, which subsequently dies (†). The parabiosis of an *ob*/*ob* and a *db*/*db* mouse leads to a lose of body mass in the *ob*/*ob* without altering the body weight of the *db*/*db*.


A
PARABIOTIC RATS
normal - normal
Lesion VMH
Food ad libitum
fat - lean
Double lesion VMH
Food ad libitum
fat - fat
Lesion
+
food restriction
normal - normal
Stimulation LH
fat - lean


B
PARABIOTIC MICE
ob/ob
fat
normal
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less fat
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fat
fat
ob/ob db/db
less fat
lean - †
fat
ob/ob → no signal
db/db → no receptor for the signal

mouse become hypophagic and died of starvation. Today we know that *ob/ob* mice are unable to synthesize leptin, while *db/db* mice as well as the obese *fa/fa* Zucker rats synthesize it, but do not respond to it due to lack of functional receptors (136).

In 1994, Friedman and associates, using positional cloning, identified and characterized the *ob* gene (258). They showed that it encodes a hormone, leptin (from the Greek leptos = thin), that is expressed mainly in white adipose tissue. The hormone informs the brain about the size of adipose stores (258). In 1995, the cloning of the leptin receptor (231) provided definitive evidence that a new physiological system implicated in body weight regulation had been discovered. The necessary conditions for leptin to be considered an afferent signal in a negative feedback loop regulating energy metabolism have now been fulfilled (79): (i) It is expressed and secreted by the principal site of fat storage, adipocytes, irrespective of their location (retroperitoneal, omental, and subcutaneous), and all adipocytes are able to secrete it (146, 147, 150). (ii) It circulates in plasma in concentrations proportional to the amount of fat mass; it has been demonstrated that obese subjects have higher levels of leptin than lean individuals, and leptin levels are reduced in obese individuals who lose weight (50, 97, 151). They are also low in malnutrition and in anorexia nervosa patients (34, 74, 93). (iii) Finally, leptin acts on the CNS, regulating appetite and thermogenesis; it has been demonstrated that leptin administration up to physiological levels results in a dose-dependent decrease in body weight due to hypophagia, even causing the death of the animal (23, 96, 177). Destruction of hypothalamic areas lead to obesity and hyperleptinemia, proving that leptin levels are regulated by the result of its central action. Interestingly, the leptin-induced weight loss is due to fat mass reduction without relevant changes in lean body mass. Thus leptin appears to be the physiological regulator of energy homeostasis (Fig. 2).

GENERAL FRAMEWORK

In an integrated framework (Fig. 2), leptin is secreted into the circulation by adipocytes reflecting the amount of fat reserves. Obesity in humans is not due to the absence of this satiety factor, but rather to central resistance to its action, while malnutrition causes low circulating leptin levels. Leptin circulates in part complexed to binding proteins (218) and shows a pulsatility in plasma (144), the role or cause of which is unknown. Individual meals do not alter leptin levels, nor is leptin capable of terminating a meal by itself (50, 151). It appears then that leptin does not participate in the short-term regulation of food intake, but rather in a longer term system, probably establishing the set point for satiety (222). Leptin shows a rhythm (50, 219), with an increase in the first hours of morning (0200 h) and nadir in the afternoon period (134, 144). Rather than a circadian rhythm, the rhythm is entrained on the meal pattern, as changing the time of meals during the day without modifying the sleep pattern changes the leptin rhythm (204). After entering the CNS, leptin binds

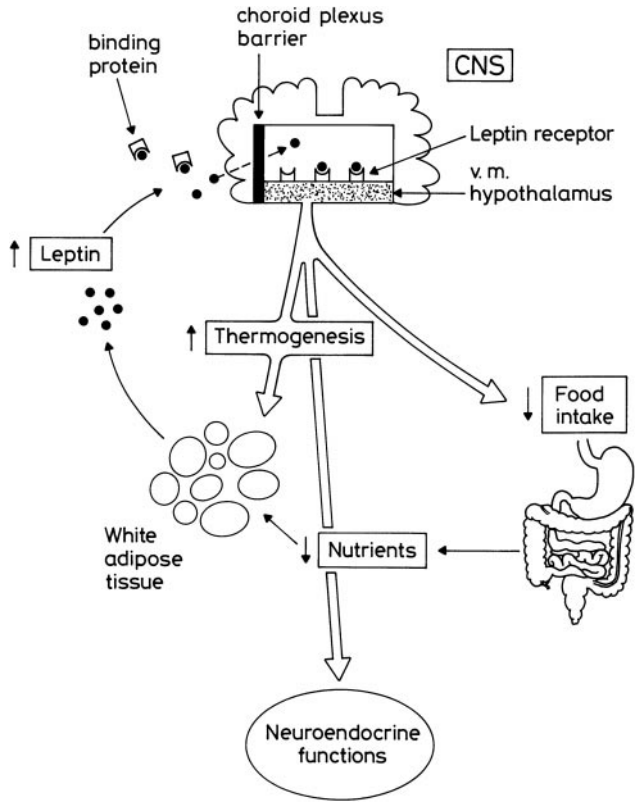


FIG. 2. General scheme of leptin physiology. Leptin is secreted from the adipocytes and circulates as free and bound forms. At both the choroid plexus and the blood–brain barrier, leptin is transported by a saturable system into the central nervous system (CNS), where it binds to specific receptors in the ventro medial (v.m.) hypothalamus. The three actions modulated by a rise in leptin are a reduction in food intake, an increase in thermogenesis, and several neuroendocrine functions over different systems.

to specific receptors, mainly in the hypothalamus, activating a complex pathway that controls energy homeostasis. Leptin excess leads to a reduced food intake plus enhanced energy expenditure through an increase in thermogenesis. Leptin reduction causes the opposite reactions, increasing adipocyte stores (Fig. 2).

Leptin has been thought of as a hormone that regulates adipose tissue stores and avoids obesity, a view which is appealing given the worldwide concern about obesity in industrialized countries. However, leptin evolved in animal and human physiology when obesity was rare. In fact, obesity is a new phenomenon, and much of vertebrate evolution took place in a setting of food shortage and famine, the common state in a hunter–hunted environment. In this setting, leptin may have evolved as a factor for maintaining energy homeostasis and the amount of fat reserves compatible with life, and as a

mediator of the adaptation to fasting (2). Therefore, rather than a fat controller, it can be argued that leptin should be viewed as a fat reporter (250).

PHYSIOLOGY OF LEPTIN

In accordance with its role, leptin is mainly expressed in white adipose tissue, irrespective of its location (91, 123, 158). The leptin gene is on chromosome 7q31.3 in humans, and its DNA has more than 15,000 bp, containing 3 exons and 2 introns that encode a 167-amino-acid secreted protein. This protein has been preserved very well throughout evolution, as mouse and human leptin share 84% homology (258). The 5′-flanking region of the leptin gene contains several putative binding sites for transcription factors such as glucocorticoid and c-AMP responsive elements, as well as the elements necessary for a constitutive secretion (91). All adipocytes are capable of secreting leptin, and it has been postulated that larger adipocytes secrete more than smaller ones (150, 158). It has recently been postulated that leptin release parallels energy influx toward the adipocyte more than cell volume or stretching (153, 235) and may be regulated by a nutrient-sensing pathway (246). However, the precise mechanism that couples adipocyte changes with leptin release is unknown (25). Adipocyte release of leptin is directly regulated by hormones and regulatory factors. For example, glucocorticoids (30, 49, 157, 170, 243), estrogens (30, 170, 215), and insulin (191, 192, 243) stimulate leptin secretion *in vitro.* However, androgens, (184), drugs able to increase intracellular c-AMP (30, 60, 220), β3-adrenergic receptor agonists (47), and phorbol esters such as PMA (183) directly inhibit leptin secretion. It is most interesting that, depending on their location, adipose tissues respond differently to these regulators; for example, subcutaneous adipocytes respond more to insulin and less to glucocorticoids than omental adipocytes (201). Furthermore, male adipose tissue responds poorly to steroid hormones compared with female tissue (30, 184). New factors such as cytokines and new antidiabetic agents, the thiazolidinediones, alter leptin expression and secretion (156, 203). This implies that leptin concentrations in plasma are determined mainly by the amount of adipose reserves, but in addition, a smaller, but significant portion of that concentration reflects a complex regulatory action exerted directly upon adipocytes by factors not necessarily related to energy homeostasis. Low levels of leptin expression in placenta (159, 213) and gastric epithelium (6) have recently been demonstrated, but the relevance and physiological meaning of these findings await further testing.

After secretion, leptin circulates in plasma in free and bound forms. It is assumed that the binding protein is a soluble form of the leptin receptor (113, 123, 218), but other alternatives are under investigation. The half-life of leptin in humans is long, approximately 75 min (110). It is assumed that the short isoform of the leptin receptor, present in kidney and lungs, mediates its clearance (55), while the forms present in several peripheral tissues such as muscle and small intestine, as well as the leptin-binding protein, delay it (110).

The exact mechanism of leptin transport into the CNS is unknown. Active leptin uptake has been described in the capillary endothelium and microvessels of brains from humans and mice, suggesting the participation of short isoforms of the leptin receptor (8, 90). However, there are some neurons with leptin receptors which project to the median eminence (95), a region that is located outside the blood–brain barrier (BBB). In any case, experimental evidence indicates that leptin exerts its action after crossing the BBB, since leptin administered by the intracerebroventricular (icv) route reduces food intake at doses that are ineffective via intravenous injection. Importantly, the effect on energy expenditure is unrelated to the route of administration of leptin (79). The transport of leptin into the choroid plexus is saturable (8, 24, 208); the existence of a threshold level of serum leptin has been suggested, above which a dissociation occurs between the serum and cerebrospinal fluid concentrations (24).

After its transport through the BBB, leptin binds to specific receptors in CNS structures, mainly in the hypothalamus. The leptin receptor is a member of the class I cytokine family which includes interferon, interleukin-2, and growth hormone (GH) receptors (231). Genetic mapping of the mouse gene encoding the leptin receptor (Ob-R) showed that it is localized on the chromosome 4 that contains the *db* locus, and different isoforms and receptor mutations have been described in rodent models of obesity. The Ob-Rb (also known as Ob-RL) is the only isoform with all the protein motifs: (a) an extracellular domain, thus capable of binding to leptin; (b) a short transmembrane region; and (c) a cytoplasmic region, capable of signal transduction. This means that the Ob-Rb receptor is most probably the only one capable of exerting the effects of leptin on body weight. This receptor has the common signal transducing subunit of a group of cytokine receptors such as interleukin-6 (IL-6), leukemia inhibitor factor (LIF), and ciliary neurotrophic factor (CNTF). The other four isoforms are generated by alternative splicing of the common leptin mRNA precursor; three have extracellular and transmembrane domains and are considered instrumental for transport. One type is truncated before the membrane-spanning domain and probably acts as a leptin binding protein in plasma (136, 142, 231). As it has been reported that the Ob-Ra short form may signal through the MAP kinase pathway (251), whether the short receptor isoforms are able to transduce the signal is still an open question. The leptin receptor is expressed in the CNS in different nuclei involved in food and body weight regulation (53, 73, 162). In particular, leptin receptor mRNA is densely concentrated in the arcuate nucleus, with lower levels in the ventromedial and dorsomedial hypothalamic nuclei (210) (Fig. 3).

The presence of leptin receptors in other areas such as endothelial and T cells, small intestine (149), and hemopoietic cells (83) suggests other actions for this hormone. There is a recent report showing that endothelial cells express the leptin receptor and that leptin triggers angiogenesis in experimental animals and *in vitro* (217).

Leptin mediates its intracellular signalling by a member of the Janus family of protein kinases (JAK), specifically increasing the hypothalamic expression of

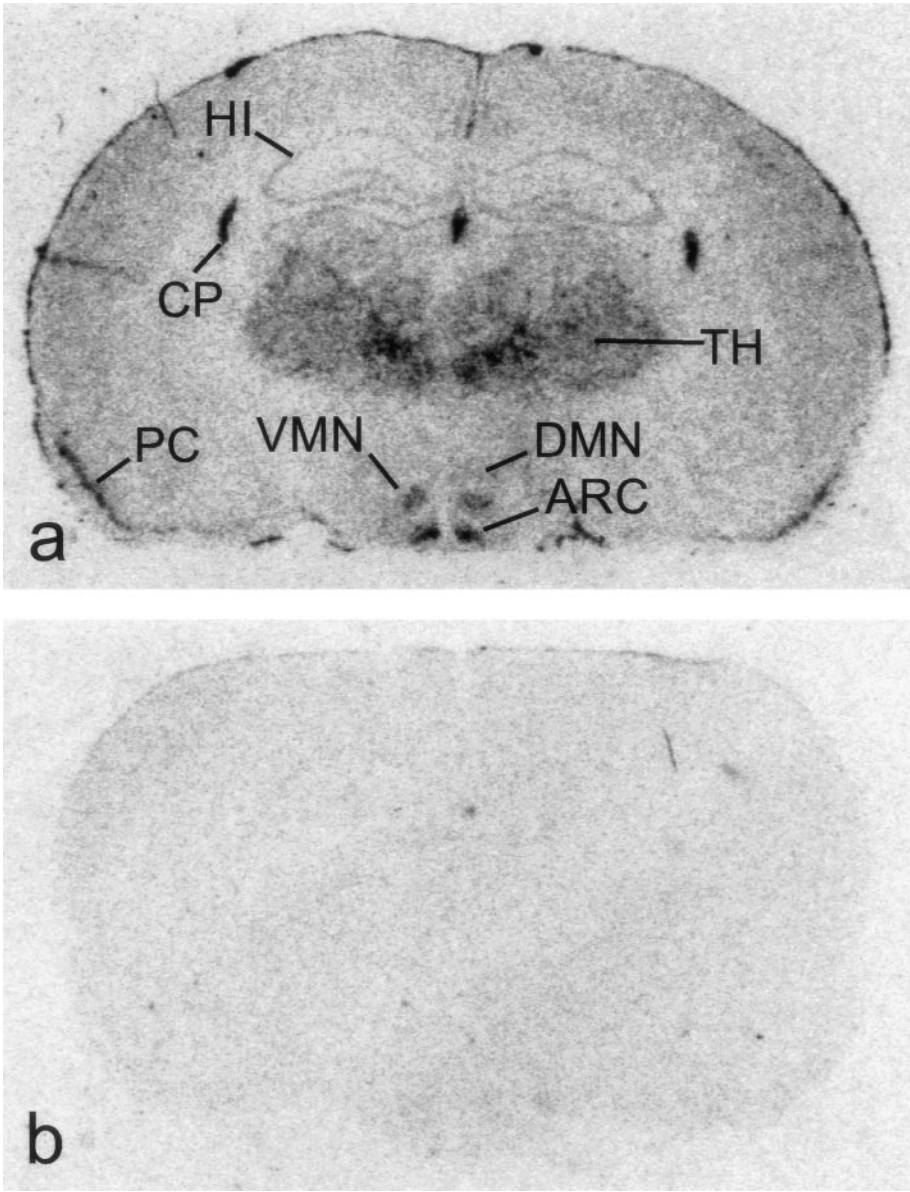


FIG. 3. *In situ* hybridization of leptin receptor mRNA in rat brain in a coronal section at the midregion level of the hypothalamus. ARC, arcuate nucleus; VMN, ventromedial hypothalamic nucleus; DMN, dorsomedial hypothalamic nucleus; CP, choroid plexus; HI hippocampus; TH, thalamus: PC pyriform cortex. From (210) with permission of the publisher.

STATs 3, 5, and 6 (5) and its target *fos* as well as other incompletely characterized genes (249). The result of this action is the alteration in neuronal synaptic transmission in hypothalamic nuclei, changing the activity of hypothalamic neuropeptides through pathways that are not yet understood (89). Neuropeptide Y (NPY) has been to date the best-studied target of leptin. Leptin reduces NPY activity shutting off activity of this stimulator of food intake leading to a reduction in food intake and increase in sympathetic tone and energy expenditure (207, 209). Other peptides regulated by leptin are orexins, CART, α-MSH, CRH, GLP-1, MCH, CCK, and a complex array of other peptides involved in the regulation of food intake (74, 77). The action of leptin at the hypothalamus must obviously be modulated by local and peripheral signals (180). This is a field of intensive research, and it is now assumed that groups of hypothalamic neurons sense rising or declining levels of leptin, eliciting an adaptive response that maintains fat deposits. As previously mentioned, the efferent signals are mainly appetite and thermogenesis. In any case, the presence of leptin receptors in CNS areas a priori not involved in body weight regulation, such as cortex, pyriform cortex, cerebellum, hippocampus, and thalamus, suggests a broad action for leptin that clearly exceeds energy homeostasis regulation.

METABOLIC ACTIONS OF LEPTIN

Leptin in plasma correlates mainly with the adipose tissue stores, but it is not a simple relationship. Leptin levels increase in parallel to the volume of adipose stores, along a curvilinear path, suggesting that leptin increases exponentially with increasing fat mass (50). Fasting reduces leptin levels, but in a way that is not proportional but exceeds the reduction in fat depots. Recovery of body fat reserves under artificial nutrition is followed by a delayed recovery of leptin levels (34). Similarly, fasting women reduce leptin levels more rapidly than men (172). All these findings suggest that leptin is not merely a stoichiometric indicator of fat deposits. The reports indicating that leptin per se has a lipolytic effect on adipocytes *in vitro* (82) add to the complexity. Some families with extreme obesity due to absence of leptin production (168, 225), or mutated leptin receptors (43), have been reported; these are the human counterparts of the *ob*/*ob* and *db*/*db* mice. However, these cases are exceedingly rare, and there is compelling evidence that obesity in humans is usually due to leptin insensitivity (24, 48), even though 5% of obese human subjects show leptin levels lower than expected (151). Since no structural alterations in the leptin receptor have been observed (48), obesity in humans is reminiscent of diabetes mellitus type 2, and its pathogenesis is still unknown.

Starvation is associated with abnormalities such as hypothermia, hyperphagia, decreased thermogenesis, depression in immune function, and neuroendocrine abnormalities such as infertility (79). Since leptin administration is able to reverse the above manifestations in *ob* mice, leptin reduction in plasma has been said to be the mediator of the physiological response to starvation (2). In

fact, administration of leptin to starved animals reverses alterations in immune function, reestablishing ovulation and normalizing glucocorticoid and thyroid hormone levels (2, 148).

Insulin is able to increase leptin production and secretion by rat adipocytes *in vitro* (192, 243), although in adult obese rats, ob mRNA does not respond to insulin (57). In humans, insulin is devoid of action unless long-term infusions are performed and supraphysiological doses administered (25, 189, 203, 242), or the patients have insulinomas (187). It is not known whether this reflects a physiological regulatory mechanism or merely a trophic effect upon the adipocyte exerted by the insulin–glucose pair. Serum leptin levels correlate with fasting insulin concentrations (180), and a positive relationship between insulin resistance and hyperleptinemia has been found (212). Data on the action of leptin on insulin secretion are conflicting, although mostly inhibitory actions have been described (67, 72, 126, 214, 228). It has recently been postulated that the inhibitory action of leptin on insulin secretion may well be mediated through the sympathetic nervous system (166). More work is needed to clarify the physiological role of leptin on insulin secretion, if indeed it has a role by itself.

States of high energy output, such as heavy exercise, are expected to be associated with changes in leptin levels that induce food intake and reduce thermogenesis. Several studies were able to detect exercise-associated leptin reductions, only after evident modifications in body composition (108, 124, 178). Recently, we reported a decrease in circulating leptin levels after a marathon run (135), suggesting that acute expenditure of high levels of energy reduces leptin values.

Under any conceptual framework, leptin appears as the neuroendocrinologically modulated hormonal mediator of energy homeostasis. This regulated system, in which leptin plays a major role, has been defined throughout the process of evolution as being so efficacious that it enabled animals to survive in a setting of food shortage and famine. In fact, the heterozygous mice either *ob*/+ or *db*/+, survived prolonged fasting significantly longer than normal homozygotes +/+, suggesting that the mutant obese genes *ob* and *db* confer metabolic efficiency (45). Nowadays, the efficacy of these "thrifty genes" responsible for leptin action in a new human setting of food abundance is generating an epidemic of obesity with tremendous implications for morbidity. The thorough understanding of how this regulatory system works would permit its control and medical manipulation in the near future.

LEPTIN AND REPRODUCTIVE FUNCTION

One of the earliest findings in the field of leptin was the remarkable gender differences in circulating leptin levels, with leptin concentrations in females being nearly twice as high as those in males, even when matched by BMI or amount of fat reserves. These findings suggest a strong relationship between leptin physiology and gonadal function in humans (101, 202).

Intrauterine and Perinatal Stage

In an ontogenic analysis it has been demonstrated that leptin is detectable in fetal cord blood as early as in the 18th week of gestation, with a dramatic increase at week 34 (99, 115). This is consistent with the onset of adipose tissue development during the second trimester of gestation and its exponential increase in the last weeks of gestation (20). Leptin levels correlate with the amount of fat in the children studied, but not with maternal serum leptin values, indicating that fetal leptin synthesis and secretion could be dependent only on the fetus (115). Whether leptin merely reflects changes in adipose stores or has a more complex role in embryonic proliferation (226) is at present unknown, but the latter possibility is supported by the detection of leptin gene expression in a variety of murine fetus tissues, such as cartilage, bone, brain, hair follicles, etc. (112). At birth, neonatal leptin levels become reduced in comparison with the previous levels, but some findings are relevant: (a) cord leptin levels in female babies are 40% higher than in males, indicating that the gender differences reported are present before birth (161, 233); (b) leptin levels at birth are disproportionally high in the neonates, as children had 5% of the body weight of their mothers but 50% of their serum leptin (233); (c) in humans, neonatal leptin levels may predict subsequent weight gain in infancy (174). As no arteriovenous differences were reported in cord blood, it is unlikely that the placenta contributed significantly, and leptin levels are most probably dependent only on the fetus (100, 130). The disproportionately high leptin/body weight ratio observed immediately after birth is normalized toward adult values 2 weeks postpartum, in both rodents (61) and children (206).

Puberty

That human reproduction, more precisely female reproduction, is linked to body fat has been known since the Stone Age, when symbols of female fertility were always depicted as fat. Populations from nonindustrialized societies know that only women with adequate amounts of fat reserves are able to get successfully through pregnancy and lactation, and in these societies, unlike our own, obese women were and are more attractive to males than lean ones. The reason for this link between body fat and reproduction is the tremendous energetic drain of pregnancy, which requires 50,000 to 80,000 Kcal to produce a viable infant, and after that, around 1000 Kcal/day for lactation (80). Hence, from a teleological point of view, it would be adequate for initiation of reproductive capability, i.e., menarche, to occur only when a given girl has built up enough adipose stores. This was the base for Frisch's hypothesis that the age at which menarche occurs is more closely related to body weight than to chronological age, adipose tissue being the determinant factor (80, 81).

Epidemiological studies support the connection between fat deposits and gonadal function, as in wealthy societies menarche appears earlier, and taller and heavier girls have menarche earlier than their counterparts (224). On the

other hand, a negative calorie balance such as that in individuals experiencing wasting diseases, severe dietary restriction, or high performance sports induces a puberty delay (112). Despite the extensive inferential (56, 211) and experimental (18, 98, 122) evidence, Frisch's hypothesis was challenged in the past because of the absence of a satisfactory mechanism connecting body fat with the hypothalamus. Leptin is such a link, and it is relevant that leptin corrects reproductive dysfunction in animal models such as the *ob*/*ob* mice that are leptin deficient and infertile (9, 35).

The hypothesis that leptin is the messenger through which adipose tissue signals the readiness to proceed into puberty to the CNS has been tested in mice. Female mice that received leptin grew at a slower rate than controls, but reproduced up to 9 days earlier (36). Leptin prevents the starvation-induced delay in ovulation in female mice (2, 37) and rats (37) as well as in severely food-restricted rats (94), thus acting as a metabolic gate for the onset of puberty. It is relevant that leptin did not advance the onset of puberty beyond that occurring in animals fed a normal laboratory diet *ad libitum* (37), suggesting that leptin is not a trigger of, but rather a permissive factor in, the complex process of puberty. It is apparent that leptin and body fat are crucial in females, but are only minimally relevant for puberty or reproductive performance in males (19).

Since no pharmacological interventions in the leptin axis are currently allowed in humans, the role of leptin in human puberty has been assessed via descriptive studies (42, 84, 155). When serum leptin concentrations were plotted in a chronological manner in 343 healthy girls and 446 healthy boys, a striking divergent pattern was observed (84) (Fig. 4). In the girls, leptin levels increased progressively in an orderly age-related way, in parallel with body weight. The leptin/body fat ratio was similar to that of adults (135). In boys, leptin levels were always lower than in girls, from the 5-year period until the end of the study. From 5 until 10 years, leptin increased, in parallel with body weight, but after that leptin levels fell even though weight continued to increase (84, 196). When leptin levels were correlated with LH, FSH, estradiol, or testosterone and puberty stages, it become apparent that changes in leptin occur mostly before puberty (84). It is relevant in this regard that there is an inverse correlation between leptin levels and age at menarche in women (160). Thus the data suggest that leptin plays a relevant role in human puberty.

The suggestion that leptin is the trigger of puberty (155) has not been supported by experimental observations in primates (185, 237) and rats (2, 61). Furthermore, our large study on pubertal development in children of both sexes showed that leptin rose progressively, reflecting adipose stores, and a brisk leptin surge was never detected (84). As a matter of fact, there is no conceptual need for a trigger; a permissive factor is all that is required. In view of this, the absence of a clear-cut leptin rise before puberty is not conclusive proof that leptin does not exert a role in puberty; a rise attaining the permissive level could occur sometime before puberty in monkeys and humans or even after birth in the rat. The only direct demonstration of a physiological role for leptin in puberty would be eliminating leptin action. This was effected by administer-

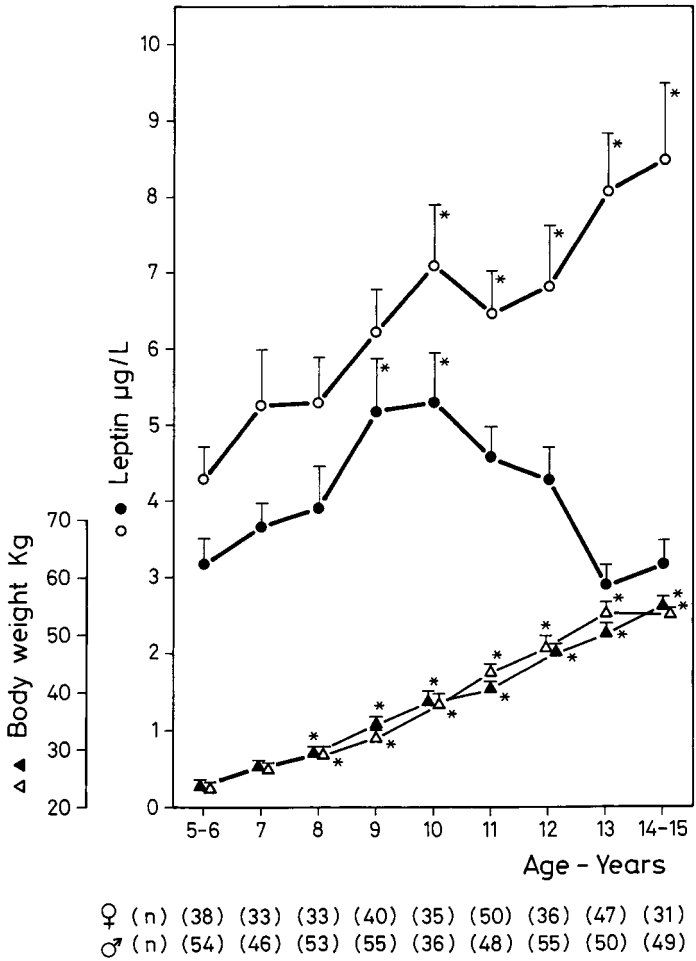


FIG. 4. Mean ± SEM leptin levels in normal children of both sexes grouped by age and compared with the increase in body weight. Leptin levels in girls paralleled body weight increase at all times and were higher than those in boys. In boys, leptin levels followed a similar pattern from 5 until 10 years old. Thereafter, there was a progressive reduction in leptin despite the continuing gain in body weight. White symbols represent girls; black symbols represent boys. The number of children in each group is in parentheses. *$p < 0.05$ vs the 5–6 year group. From (84) with permission of the publisher.

ing leptin antibodies icv in experimental animals, and a delay in vaginal opening occurred (E. Carro, C. Dieguez, F.F. Casanueva, unpublished). This result indicates that leptin regulates puberty at the hypothalamic level and not via direct gonadal activation. The presence of functional leptin receptors in the hypothalamus (162) and its action upon GnRH release (152, 253) support this view. In any case, the most direct proof for a role of leptin in human puberty comes from the families in which leptin action was not operative due to mutation of either the leptin gene (225) or the leptin receptor (43). In these

patients, puberty was absent, and there was a state of hypogonadotropic hypogonadism.

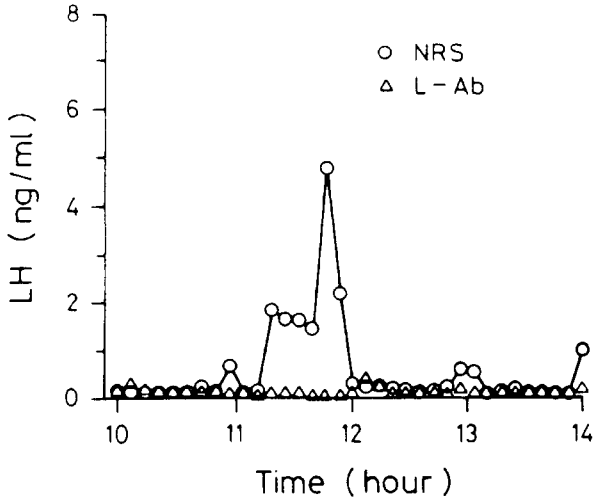
In conclusion, there is a wealth of epidemiological, inferential, clinical, and experimental evidence suggesting that by delivering leptin, adipose tissue signals to the CNS the adequacy of the energy stores, which permits sexual maturation to occur. Leptin seems to act as a permissive factor for puberty.

Adult Gonadal Function

In healthy women, serum leptin levels are at least two times higher than in men, and they decline after menopause (199). During the menstrual cycle, minor or no changes in leptin levels have been reported (193). These descriptive studies provide no direct answer to the question of whether leptin participates in gonadal regulation. The first demonstration of a relevant leptin role in experimental animals comes from our studies after immunoneutralization of leptin action at the hypothalamic level by administering leptin antibodies icv, i.e., inside the BBB (26). Twelve hours after the first administration of leptin antibodies in this fashion, rats lose LH pulsatility, and the estrous cycle stops in anestrus, without a recurrence of cycles throughout the 8 days of the study (26) (Fig. 5). In very elegant studies, it has been demonstrated that leptin stimulates or at least exerts a permissive action over GnRH release from the hypothalamus (152, 253) and stimulates LH and FSH release from the pituitary *in vitro* (253). In conclusion, although direct experimental evidence is lacking in humans, leptin seems to exert a facilitatory role in the functioning of the GnRH–gonadotropin axis.

Role of Leptin in the Alterations of Gonadal Function Mediated by Undernutrition and Exercise

The importance of energy stores in maintaining fertility is best demonstrated in the menstrual cycle dysfunction commonly found in women who lose weight below a certain threshold level (116). Such women have low plasma levels of estrogen, LH, and FSH, with a circadian pattern of LH secretion similar to that of prepubertal girls (17). In fact, a classical statement regarding patients with anorexia nervosa is that they revert to the prepubertal pattern of gonadotropin secretion. A delay in puberty is seen in girls who are undernourished before puberty (179), and marked delays in the onset of puberty occur in nutritionally deprived animals, with rapid recovery of reproductive maturity when food is available *ad libitum* (78). Similarly, women undertaking chronic severe exercise develop the so-called exercise-induced amenorrhea, ascribed to loss of weight, low percentage of fat, increased expenditure of energy, or decreased energy intake, with the consequent loss of pulsatile LH secretion from the anterior pituitary (241). This is rapidly resolved when exercise ceases. The findings suggest that "metabolic stress" with or without weight loss may affect the reproductive axis and more severely in women (116).



NRS	0	1	2	3	4	5	6	7	8	9	10
1	○	●	○	○	○	●	○	○	○	●	○
2	○	●	○	○	○	●	○	○	○	●	○
3	○	●	○	○	○	●	○	○	○	●	○
4	○	●	○	○	○	●	○	○	○	●	○
5	○	●	○	○	○	●	○	○	○	●	○
6	○	●	○	○	○	●	○	○	○	●	○

L-Ab	0	1	2	3	4	5	6	7	8	9	10
1	○	●	○	○	○	○	○	○	○	○	○
2	○	●	○	○	○	○	○	○	○	○	○
3	○	●	○	○	○	○	○	○	○	○	○
4	○	●	○	○	○	○	○	○	○	○	○
5	○	●	○	○	○	○	○	○	○	○	○
6	○	●	○	○	○	○	○	○	○	○	○
7	○	●	○	○	○	○	○	○	○	○	○

FIG. 5. Freely moving female rats were injected icv with either leptin antibodies (L-Ab) or normal rabbit serum (NRS) as control. The immunoneutralization of leptin at the hypothalamic level with the antiserum blocked the normal pulsatility of luteinizing hormone (LH). (Bottom) Black dots represent the day of estrus in normal cycling rats, given NRS or leptin antibodies. The leptin antibodies completely and immediately produced anestrus. From (26) with permission of the publisher.

Leptin may be the link between this metabolic stress and the GnRH pulse generator (152). Women with severe undernutrition due to anorexia nervosa show extremely low levels of leptin in plasma (34, 93) and lose the circadian rhythm of leptin (7). The low leptin levels correlate with the reduction in adipose stores, but are unrelated to the type of nutritional disease involved (74). Interestingly, when anorectic patients partially recovered body weight by artificial nutrition, leptin values showed a delay in recovery in comparison with fat mass (34). In patients with anorexia nervosa, cerebrospinal fluid (CSF) leptin correlates with the nutritional status, but the CSF to plasma leptin ratio is higher for patients than for controls and normalizes before body weight is normalized (154). It may be hypothesized that differences in the recovery of leptin levels may be the basis for the continued absence of menses in some patients after body weight normalization. In trained athletes, the low leptin levels observed merely reflect the extreme reduction in fat stores produced by the intensive training (108, 133, 135).

Since leptin enhancement or reduction by pharmacological intervention is not permitted in humans, the proof that leptin plays a relevant role in the gonadal disturbances related to undernutrition or extreme exercise must be sought in experimental animals. Leptin is able to prevent the reduced pulsatile LH secretion that occurs during fasting in monkeys (75) and in rats (171). It also affects sexual behavior in female hamsters (244). These relevant effects of leptin are complex, considering the hypothalamic distribution of leptin receptors and implicate neuronal systems such as NPY and POMC (75). However, there is a redundancy in the participation of NPY, as the NPY knockout mouse is fertile and also maintains normal body weight (69).

Direct Interaction between Leptin and Gonadal Function

Although the existence of gender-based differences in serum leptin levels is undisputed, the mechanism explaining them is unknown. Gonadal steroid hormones act directly on white adipose tissue, estrogens being effective leptin releasers with *in vitro* potency similar to glucocorticoids (30). Androgens are weak and inconsistent suppressors of leptin secretion *in vitro* (184). Interestingly, both estrogens and androgens were effective in omental adipose tissue from women donors, while male samples were minimally affected by these steroids. This may explain the reported gender differences in plasma leptin concentrations. Although androgens have been reported as leptin suppressors (64, 118), this action is evident only after changes in body composition. In human adipose tissue *in vitro,* testosterone exerts a slight stimulation of leptin secretion that is most probably the net result of a stimulatory effect by its partial aromatization to estradiol, minus a smaller inhibitory action mediated by its transformation in DHT (184).

Direct actions of leptin on gonadal activity have been reported. A direct intraovarian effect of leptin antagonizing the action of IGF-I on estradiol and progesterone production was reported in granulosa cells (221, 255) and recently

confirmed in human ovarian tissues (1). The effect was probably mediated by specific leptin receptors in the ovary (40, 119). Similarly, direct action on testicular steroidogenesis has been noted in rats (232). This multiloci action of leptin suggests that energy homeostasis plays in the hypothalamic–pituitary–gonadal axis a more relevant role than previously thought. Future research in this area should clarify the physiological relevance of these pharmacological findings.

Pregnancy

Pregnancy is a hypermetabolic state in which a great increase in maternal body fat and weight occurs, mostly in the final trimester of gestation (104), and it is associated with relevant neuroendocrine changes as adaptation to the new hormonal status. Hypermetabolic changes occur also in the fetus (4). There is no increase in energetic efficiency in pregnancy, and the energy balance becomes positive primarily due to an increase in food intake, which is necessary to prevent the depletion of maternal energy stores (195). In humans and some other species, maternal fat accumulates during gestation and is used during lactation. To determine which of the significant changes in appetite, thermogenesis, lipid metabolism, and neuroendocrine adaptations may be produced by leptin, this hormone has been measured in several animal models and in humans. There is a general and undisputed pattern of leptin change, i.e., leptin increases at least twofold at midpregnancy, followed by a decrease just before parturition (Fig. 6). As it has been observed in rodents (38, 234), and confirmed in gestational women (21, 130, 159, 205), leptin increases in the first trimester of pregnancy, before any major changes in body fat and resting metabolic rate (109, 130). The increase is also unrelated to fetal growth (227). Immediately before delivery, leptin levels undergo a dramatic drop, returning to the levels in nonpregnant women 24 h before delivery, and are further reduced 24 h after delivery (130). At least in rodents, the higher leptin levels are, in fact, a complex of leptin and leptin binding protein (86). However, there is no clear explanation of the role of the increased leptin in human pregnancy or the mechanism for these changes.

The role of leptin in pregnancy has been addressed by mating the leptin-deficient and sterile *ob*/*ob* female and *ob*/*ob* male mice, treating them with exogenous leptin, and then stopping leptin administration at different intervals after mating. In this way, they obtain a model of pregnancy with different intervals of absence of circulating leptin in both mother and fetuses (169). The absence of leptin did not hamper implantation and development of the fetus, nor gestation and parturition, but leptin resistance was observed in the pregnant mother at mid-gestation, probably from a desensitization of leptin receptors or the intracellular signalling (169). A feasible teleological explanation for that observation is the need to maintain in the mother an increased

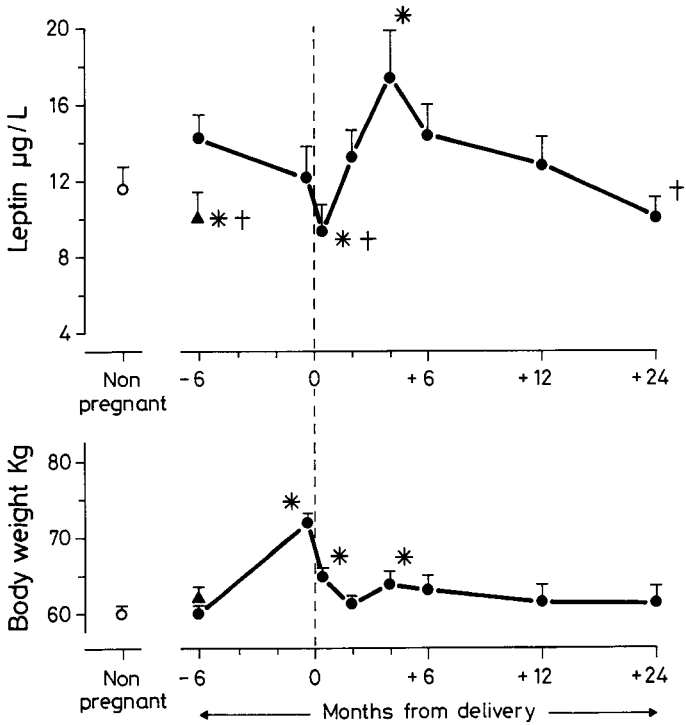


FIG. 6. Black dots represent the mean ± SEM of serum leptin levels and body weight in a group of women transversally studied throughout pregnancy and postpartum. A rise in leptin was observed during pregnancy followed by a reduction before and after delivery. Afterward, a progressive increase over the first 6 months postpartum was observed. All these changes in leptin levels were unrelated to body weight changes. Black triangles represent the serum leptin and body weight in a group of women who suffered spontaneous abortion in the first trimester of pregnancy. *$p < 0.05$ vs nonpregnant group, †$p < 0.05$ vs −6 months group. From (130) with permission of the publisher.

food intake despite the already increased adipose depots, to continue the build-up of nutrient reserves for lactation.

It is not certain which tissue produces the elevated levels of leptin in pregnancy. As human placenta is a source of leptin (159, 213), it has been suggested that maternal leptin levels are derived from this organ. During pregnancy in rodents, serum leptin levels increase 25 times in mice and twofold in rats and are detectable and possibly synthesized in the placenta (12, 111, 120). The placenta also expresses the leptin receptor (111), indicating that the placenta itself may be a target for leptin action. In humans, it is not known whether the circulating leptin in pregnancy comes from the placenta or from adipose tissue. However, there are indirect suggestions that the placenta plays a major regulatory role, since placental pathology is associated with variations in leptin levels (141, 159, 164). Furthermore, there is a rapid decline in circulating leptin when placental function vanishes such as at parturition and in spontaneous abortion (130). In any case, the human placenta is a complex

organ with a variety of encoded and expressed neuroendocrine systems; therefore, the presence of leptin with specific regulatory systems (12, 254) would not be surprising.

After delivery and the immediate normalization of leptin levels, a progressive rise in serum leptin levels was observable in the following weeks. These high leptin levels remain elevated throughout the first 6 months postpartum and decreased thereafter (130) (Fig. 6). These changes did not reflect any variation in BMI in women nor were they obviously influenced by the fetus or placenta and they are unrelated to the mother's lactation. Rather than leptin reflecting fat stores, it seemed that body weight followed the previous leptin rise. The 6 months after partum in which the mothers had been exposed to abnormally high levels of leptin may well have raised the set point of the hypothalamic leptin receptor through desensitization. This is an unproved, but attractive hypothesis for explaining postpartum weight retention or postpartum weight increase described by some women. The neuroendocrine mechanisms explaining these leptin changes await further study.

Leptin in Human Milk

True human leptin has been purified and identified in milk and colostrum from nursing mothers (31), a relevant finding considering that milk replaces the placenta in providing the newborn with critical nutrients and growth factors. Although a small portion is transferred to milk from the circulation, leptin may be mostly synthesized in ductal epithelial cells. With regard to its potential role, it is worth mentioning that in rats, leptin is transferred from the mother's milk to the pup's stomach and then to the nursing pup's serum (31). Leptin is another large protein that in the neonatal period may be absorbed without degradation by the intestinal system, suggesting that it may play a role in the regulation of neonatal food intake, in intestinal maturation, or in the responsiveness of the pup's adrenal axis to stress (31, 236). On the other hand, lactation suppresses the diurnal rhythm of serum leptin, inducing relative hypoleptinemia, which may be a factor in promoting the hyperphagia of lactation (181).

Nonfertile Stages

Although a considerable amount of controversy surrounds this issue, no leptin increase is observed in women with polycystic ovarian syndrome; elevated levels might be expected because of the increased adiposity and steroid hormone imbalance (132). The reduction in leptin levels observed in aging or in menopause reflects the reduced fat stores and the decline in steroid hormones which stimulate leptin production (167, 199).

ROLE OF LEPTIN IN GROWTH HORMONE (GH) NEUROREGULATION

Unlike other pituitary hormones, growth hormone (GH) exerts its biological actions on almost every cell of the organism and plays an important role in the control of metabolic processes (62). Several studies have shown that GH deficiency is associated with abnormalities in body composition, metabolic derangement, and suboptimal physical performance; these impairments improve with GH replacement therapy (39). In disease states such as obesity and chronic hypercortisolism with increased adiposity and/or central distribution of fat, both spontaneous and stimulated GH secretion are severely impeded. On the other end of the spectrum resides malnutrition and fasting in humans, both of which are associated with increased GH secretion when confronted with most, if not all, stimuli (62).

As the common factor in all these situations is increased or decreased adiposity, or changes in energy homeostasis, we postulated that adipose tissue exerts a relevant role in the control of GH secretion in humans through two signals, i.e., free fatty acids acting at pituitary level and leptin modulating hypothalamic activity (33). There was indirect evidence supporting a regulatory role of leptin derived from data showing that rodents and humans with mutations of the leptin gene or of its receptor exhibited reduced plasma GH levels (43, 229). Furthermore, it was found that while leptin markedly increases *in vitro* GH secretion from fetal anterior pituitary cells at early stages of gestation, this stimulatory effect disappears in cultures of fetal human anterior pituitary cells at the end of gestation (Fig. 7) (216). By bypassing the blood–brain barrier by the icv administration of either leptin or receptor antibodies, we provided the first evidence that leptin regulates GH secretion (27, 190).

Hypothalamic Regulation of GH Secretion by Leptin

We assessed the role played by leptin in regulating GH secretion by administering leptin antiserum, icv, to normally fed rats. This procedure led to a clear-cut decrease in plasma GH levels, indicating that physiological leptin levels are needed to ensure normal spontaneous GH secretion (27). Since fasting is associated with a marked decrease in circulating leptin levels (2), we thought that the decrease in spontaneous GH secretion in this animal model could be leptin mediated. As expected, 48 h following food deprivation there was a suppression of spontaneous rat GH secretion (27), and we found that following acute administration of leptin icv there was a reversal of that inhibitory effect on both spontaneous GH secretion and GH responses to GHRH (Fig. 8) (27). On the other hand, in rats fed *ad libitum,* and therefore with normal leptin levels, icv leptin administration acutely or by infusion for 3 days failed to modify spontaneous GH secretion (27, 240). A 7-day icv infusion increased GH secretion (230); however, to interpret results coming from long-term icv administration, it should be considered that leptin efflux from the cerebrospinal fluid

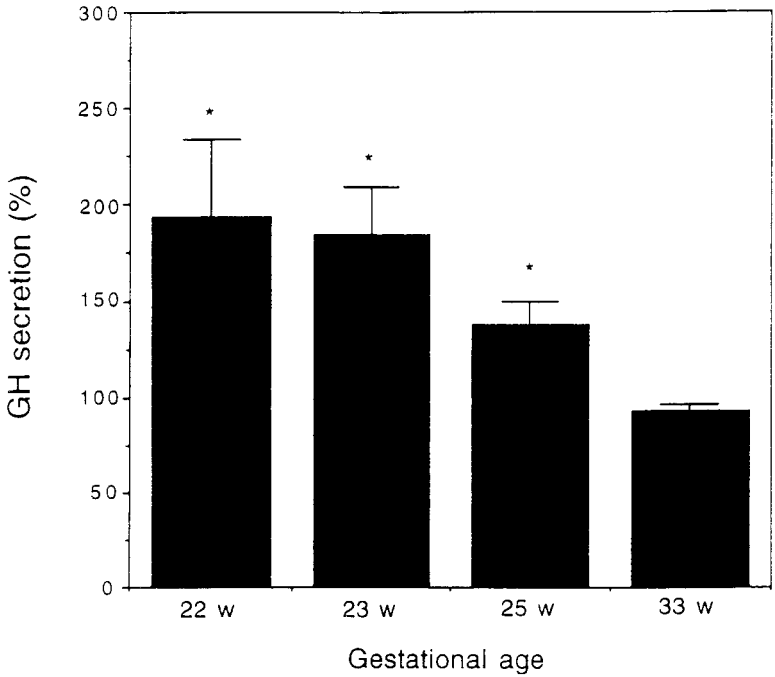


FIG. 7. Stimulatory effect of leptin on *in vitro* GH secretion from fetal anterior pituitary cells was observed at earlier times of gestation (22, 23, and 25 weeks) but not at a later stage (33 weeks). *$p < 0.05$. From (216) with permission of the publisher.

to blood is so intense (153) that reported leptin actions may be peripheral as well as central. Taken together, these data suggest that normal circulating leptin levels exert a maximal effect on GH secretion, and only chronic sustained hyperleptinemia is able to further enhance GH secretion. On the other hand, the fact that exogenous leptin administration reversed fasting-induced suppression of spontaneous GH secretion indicates that the low circulating leptin levels present in rats after food deprivation may well be the main factor responsible for their impaired GH secretion. Intracerebroventricular administration of leptin markedly increased *in vivo* GH responses to exogenously administered GHRH in fasted rats (E. Carro, R. Seoane, F.F. Casanueva, and C. Dieguez submitted for publication), indicating that the hypothalamic actions of leptin are translated to the pituitary functionalism.

Although the mechanisms by which leptin exerts its effects are far from being completely understood, the presence of leptin receptors in different hypothalamic nuclei, including the periventricular and arcuate nuclei of the hypothalamus (210) where somatostatin and GHRH neurons are located, suggests an action of leptin at the hypothalamic level on both somatostatin and GHRH gene expression. Using double-labeling immunofluorescence histochemistry it was found that a small population of GHRH producing neurons in the arcuate nuclei express leptin receptors (95). Our finding that passive immunization

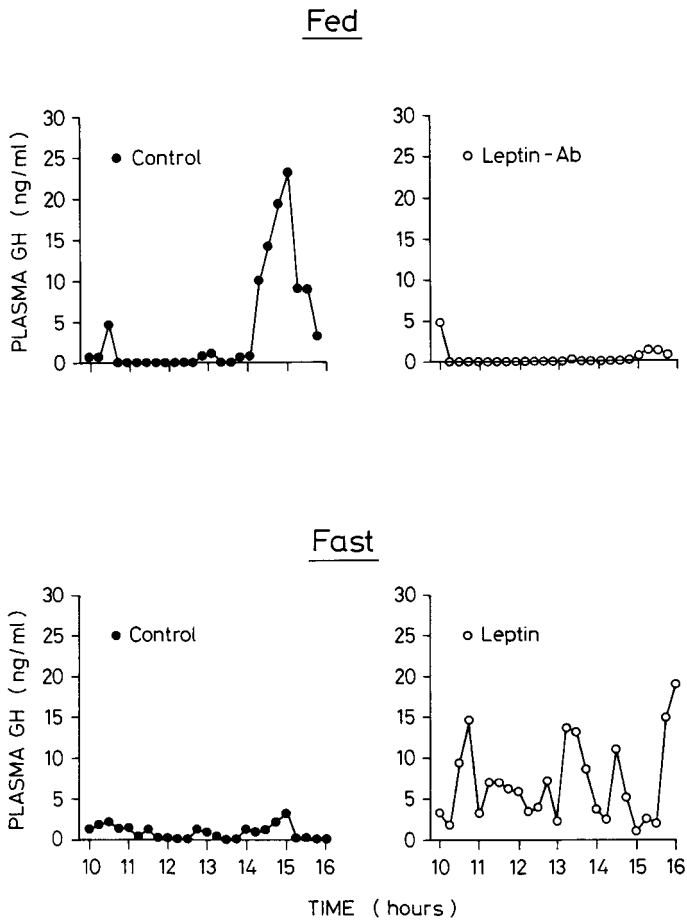


FIG. 8. (Top) Representative plasma GH profiles in individual freely moving male rats after icv injection of normal rabbit serum (control) or leptin antibodies (L-Ab). (Bottom) Administration of vehicle or leptin in freely moving fasted rats. Redrawn from (27) with permission of the publisher.

with anti-GHRH serum completely abolished the stimulatory effect of leptin on GH release in food-deprived rats demonstrates that the leptin actions are partially dependent on GHRH (28). Whether this action of leptin is exerted through GHRH or merely requires the presence of GHRH tone could not be distinguished. As the fasting-mediated decrease in GHRH mRNA levels in the arcuate nucleus was completely restored by leptin administration, these results indicated a role of GHRH as a mediator of the leptin actions on GH secretion (28).

Leptin administration to monolayer cultures of fetal rat neurons has been found to inhibit basal and forskolin-stimulated somatostatin secretion and somatostatin mRNA levels (190). Further studies carried out in perifused adult rat hypothalami and *in vivo* also supported a leptin action at the hypothalamic level via somatostatin. The fact that both leptin administration and passive

immunization with antisomatostatin serum restored GH secretion in food-deprived rats provided circumstantial evidence that leptin-induced GH secretion could be mediated by a decrease in somatostatin release from the hypothalamus (28). In support of this possibility it was found that leptin decreased somatostatin mRNA levels in the periventricular nuclei of fasted hypophysectomized rats (28). However, whether the effects of leptin on GHRH and somatostatin-producing neurons are exerted directly or through other neuropeptides is still unclear. Our own data support the second possibility, since we found that hypothalamic NPY mediates some if not all leptin actions on GH regulation (29, 240). Further studies looking at the effects of leptin on GH secretion in NPY knockout mice should help to clarify this matter. Finally, these actions may be species specific as a stimulatory effect of leptin on GH secretion has been reported in pigs but not in sheep (10, 103).

Pituitary Actions of Leptin Regulating GH Secretion

The membrane spanning leptin receptor and several shorter isoforms are present in the pituitary in normal adult rats, mice, and sheep, as well as in fetal human anterior pituitary tissue (216, 257). This opens the possibility of a direct action of leptin at the pituitary level. However, the effects of leptin on *in vitro* GH secretion appear to be quite complex. Thus, while in rats and sheep, short-term exposure (30 min to 4 h) to leptin did not influence basal GH secretion (197), this was increased in pig anterior pituitary cells (10). On the other hand, while long-term treatment with leptin (24 h) increased *in vitro* basal GH secretion, it inhibited GHRH-stimulated GH secretion from both ovine and pig cells (10, 197). At present, it is unclear whether the leptin receptor long isoform is expressed in the somatotrope cell or other cell types of the anterior pituitary gland and the functional significance of the leptin actions at pituitary level on GH secretion remain to be studied. Interestingly, transgenic mice for GHRH showed an enhanced expression of pituitary leptin receptors, suggesting that either GHRH or GH may regulate leptin receptor gene expression in the anterior pituitary (22).

Interrelationship between Leptin and GH in Humans

Based on inferential evidence, it has been postulated that in humans leptin may act as an inhibitor of *in vivo* GH secretion. In fact, in disease states such as obesity or Cushing's syndrome associated with enhanced leptin levels, GH secretion is markedly blunted (32). On the contrary, in states such as malnutrition associated with leptin reduction, GH secretion is enhanced (33). Similarly, in trained athletes with low leptin levels, due to the reduction in fat mass, there is an increase in GH secretion as both a response to an acute stimulus and in the form of basal pulsatile release at rest (33). As no studies administering leptin to humans have been published so far, this putative inhibitory role of

leptin on GH secretion in humans is not supported by any direct experimental evidence. Nevertheless, it is consistent with the reports showing that serum leptin levels were inversely correlated with spontaneous GH secretion and the GH responses to GHRH in some physiological and pathophysiological settings (54, 88, 200), and with the increased disorderly GH secretion while fasting (11).

The mechanism by which leptin and GH are inversely correlated has not been determined. They may be independently regulated covariables; GH may inhibit leptin secretion by a direct action on fat cell secretion or fat mass, or leptin may inhibit *in vivo* human GH secretion. Data so far available do not support a direct effect of GH on *in vitro* leptin secretion from the adipocytes (14). On the other hand, there is some evidence that GH may influence *in vivo* serum leptin levels. Thus, in patients with acromegaly, who show decreased fat mass, leptin levels are lower even after correction for the percentage of body fat, suggesting that excess GH/IGF-I reduces serum leptin levels by reducing body fat mass and/or by other unknown mechanisms (165). In keeping with this observation, circulating leptin levels are elevated in patients with growth hormone deficiency, and long-term GH treatment results in a decline in leptin levels (3, 76, 128), indicating that the lower leptin levels observed after chronic GH administration could be due to a reduction in fat mass. In elderly subjects with GH deficiency, a high dose, single bolus of GH led to an increase in leptin in 24 h but was not related to IGF-I or insulin, and chronic treatment with GH did not change leptin values (87).

Although not a universal finding (76), a strong correlation between leptin and GH binding proteins has been found in children and adults across a wide range of nutritional states (13, 127, 145). On the basis of this association, it has been proposed that low leptin levels in undernourished patients may account for their decreased production of GHBP and IGF-I, which through reduced negative feedback results in increased GH secretion from the anterior pituitary (145). However, direct evidence to support this hypothesis is lacking at present. Further studies assessing the effects of leptin administration on GH secretion in humans are required to uncover its mechanism of action on human GH secretion. The recent finding that serum leptin levels provide a strong metabolic marker for the growth response to GH treatment in children underlines the existence of a strong interrelationship between leptin and the GH axis (128).

LEPTIN AND TSH

Alterations in thyroid hormone levels are frequently associated with changes in body weight. In a large number of hyperthyroid patients (85%) there is a decrease in body weight, whereas in 59% of hypothyroid patients there is an increase. These changes in body weight are not due to changes in food intake; on the contrary, appetite is increased in only 65% of hyperthyroid patients and decreased in just 45% of hypothyroid patients. Taking into account that leptin has been shown to decrease food intake and increase energy expenditure, the

assessment of the influence of thyroid status on serum leptin levels has been explored in different experimental settings.

Hypothyroid rats exhibited elevated leptin mRNA levels which normalized after appropriate thyroid hormone treatment (71). Similarly, assessment of leptin levels in rats with a wide range of thyroid status, ranging from overt hypothyroidism to severe hyperthyroidism, indicates that thyroid hormones exert an inhibitory influence on serum leptin concentrations (70). Nevertheless, it should be noted that severely hypothyroid rats exhibited leptin concentrations similar to those of control rats, although they were higher when leptin values were corrected for BMI. On the other hand, while hyperthyroid rats exhibited lower serum leptin concentrations than controls, these differences were smaller when corrected for body weight (70). Taken together, these data indicate that thyroid hormones exert an inhibitory effect on circulating leptin concentrations in the rat.

Studies on the effect of thyroid status on leptin levels in humans have produced conflicting results. Some studies found no effect of changes in thyroid status on leptin levels (51, 223). Some showed elevated levels (140, 182), and others showed suppressed (238, 252) leptin levels in hypothyroidism. Similarly, while few studies reported lower leptin levels in hyperthyroid patients (259), most studies failed to find any significant change in comparison to sex, age, and BMI-matched controls (51, 140, 182, 223, 238).

Although the data available are as yet scarce, leptin has emerged as a possible regulator of the hypothalamo–pituitary–thyroid axis. It is well known that food deprivation, a condition associated with low leptin levels, leads to low plasma T3, T4, free T3, and free T4 levels, as well as decreased TSH and TRH synthesis in the pituitary and the hypothalamus, respectively (175). Administration of leptin to food-deprived rats has been reported to restore to normal the decreased proTRH mRNA levels in neurons of the paraventricular nucleus (Fig. 9) (137, 138). This increase in TRH synthesis could explain the stimulatory effect of leptin on circulating TSH levels in euthyroid food-deprived rats. Finally, leptin administration to fasted rats normalized their decreased T4 and T3 (2, 137). Taken together, these data indicate that fasting-induced reduction in proTRH mRNA levels in neurons of the paraventricular nucleus can be prevented by systemic administration of leptin. Therefore decreased circulating leptin levels during fasting may act as the critical signal to TRH neurons to reset the set point for feedback regulation of TRH gene expression by thyroid hormones. This mechanism would inhibit TRH synthesis during fasting, when thyroid hormones are low, leading to decreased TSH secretion and TSH stimulation of the thyroid gland, and by reducing thyroid thermogenesis, act in co-ordinate fashion with other homeostatic mechanisms to allow adaptation.

Theoretically, the effects of leptin on TRH could be exerted directly, mediated by other neuropeptides, or mediated indirectly through its effects on other systems, such as the hypothalamo–pituitary–adrenal–axis. Recent data support the conclusion that the effects of leptin on TRH are mediated by other neuropeptides present in the arcuate nucleus, since it was recently shown that arcuate nucleus ablation, following treatment with monosodium glutamate,

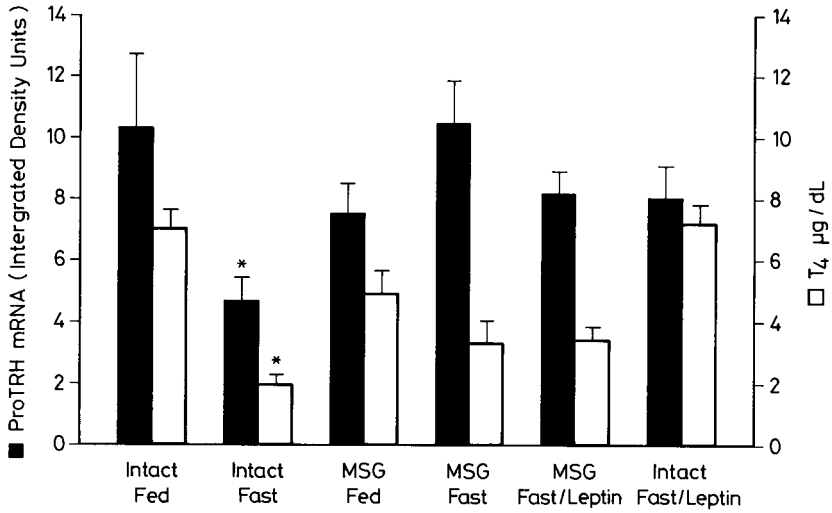


FIG. 9. Serum T4 levels (open bars) and proTRH mRNA content (black bars) in the paraventricular nucleus and serum T4 of intact fed and fasted animals receiving leptin, with or without ablation of the arcuate nucleus due to treatment with monosodium glutamate (MSG). $^*p < 0.05$. Redrawn from (137, 138) with permission of the publisher.

completely prevents fasting-induced suppression of proTRH mRNA levels in the paraventricular nucleus (Fig. 9) (138). Furthermore, the arcuate nucleus contains abundant leptin receptors and sends strong axonal projections to the paraventricular nuclei (95, 139, 210). In addition, in arcuate nucleus-ablated animals, fasting failed to reduced plasma T4 levels (138, 194). These observations indicate that during fasting the arcuate nucleus is essential for the normal responses involved in the adaptation of the hypothalamus–pituitary–thyroid axis to starvation and that the hypothalamus serves as a critical locus of leptin regulation of this axis (Fig. 9). The mechanism by which arcuate neurons mediate the effects of leptin on TRH-producing neurons in the paraventricular nuclei remains to be clarified. NPY was an obvious candidate (175), since NPY neurons in the arcuate nucleus contain abundant leptin receptors, and they project heavily to TRH-producing neurons in the paraventricular nucleus (95, 210). However, the fact that in NPY knockout mice, fasting still induces a marked fall in plasma T4 levels rules out symplistic explanations (68). The possible involvement of other neuropeptides that are synthesized in the arcuate nucleus and project to the paraventricular nucleus, such as α-MSH (143), needs to be studied. Furthermore, the possibility that leptin in addition to its action at the hypothalamic level could act directly on the thyrotrope or in the thyroid gland is still unexplored.

Whatever the mechanisms, data obtained in patients with leptin deficiency or leptin receptor mutation also support a permissive role for leptin in the hypothalamic–pituitary–thyroid axis, since some of the affected members exhibited features of hypothalamic hypothyroidism (43, 225). Additional studies assessing the effects of leptin on *in vivo* TSH secretion in both rats and humans

are eagerly awaited to gain further insight into the mechanisms by which leptin acts and its physiological role in the regulation of the hypothalamus–pituitary–thyroid axis.

LEPTIN AND THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

There is a well-established relationship between the nutritional status of mammals and the activity of the hypothalamic–pituitary–adrenal (HPA) axis. Nocturnally active rats with free access to food manifest a peak of plasma corticosterone just prior to the time of onset of predominant food intake. There is a glucocorticoid response to food intake in most species, and in humans this is particularly noticeable at lunch time. Moreover, it is clear that glucocorticoids play an important role in the regulation of long-term energy balance, and relative glucocorticoid hypersensitivity has been postulated as an etiological factor in obesity. Thus, high levels of glucocorticoids are seen in most strains of genetically obese mice, while the metabolic defects in the *ob*/*ob* mouse and other rodent obesities could be corrected by removing or blocking the effects of adrenal glucocorticoids (79). Measurement of serum leptin levels over 24 h in normal adult subjects have shown the presence of a pulsatile secretory pattern with an average of one pulse every 45 min and a pulse duration of 3.2 min (144). An inverse interrelationship between plasma levels of leptin and those of ACTH and cortisol was noted in normal subjects (Fig. 10) (144), while in patients with acute sepsis there was an associated loss of diurnal rhythm of cortisol and leptin secretion (15). The interrelationship between leptin and the HPA axis covers the actions of glucocorticoids on leptin synthesis as well as the regulation by leptin of the HPA axis.

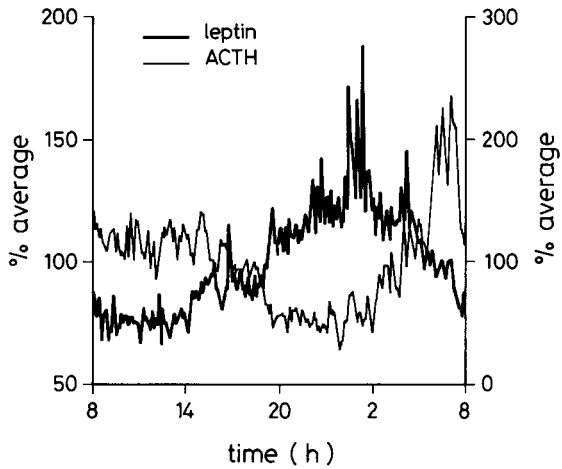


FIG. 10. Simultaneous fluctuations of leptin and ACTH levels over 24 h in normal adult male subjects sampled at 7-min intervals. A significant negative correlation between the two hormones was observed. Redrawn from (144) with permission of the publisher.

Glucocorticoid Regulation of Leptin Secretion and Leptin Gene Expression

In vitro studies of rodent adipocytes have shown that glucocorticoids increased leptin mRNA levels and leptin secretion (220). These effects appear to be due to the direct transcriptional actions of glucocorticoids on glucocorticoid responsive elements present in the leptin promoter (91). In keeping with this, *in vivo* treatment of rodents with glucocorticoids at catabolic doses produced an increase in adipose tissue leptin mRNA and serum leptin levels (Fig. 11A), whereas food intake and body weight decrease concomitantly (59). In humans, dexamethasone increases leptin mRNA levels and leptin secretion by female omental tissue *in vitro* (Fig. 11B) (30). The stimulatory effect of dexamethasone was time and dose dependent and could be inhibited by PKC activation with PMA (183). In contrast, dexamethasone failed to consistently stimulate *in vitro* leptin secretion from human omental tissue taken from male donors, thus indicating the existence of important gender differences in the regulation of leptin gene expression by glucocorticoids (30). Whether these differences are due to a differential regulation of the leptin promoter, different levels of glucocorticoid receptors, or other factors is at present unknown.

Data obtained *in vivo* have shown that pharmacological doses of exogenously administered glucocorticoids produce a sustained rise in circulating levels of leptin in normal and obese subjects (58, 131, 163, 176). Whether this glucocorticoid action is exerted at physiological or pharmacological doses is a matter of debate, as the mild hypercortisolism that follows the administration of CRH was not accompanied by an elevation in plasma leptin in normal subjects or in patients with Cushing's syndrome (235). However, it has also been shown that variations in glucocorticoid levels within the physiological range for long periods affect plasma leptin levels (65). It appears that while short-term changes in cortisol levels in the physiological range do not have an effect on plasma leptin concentrations, chronic elevations exert a marked stimulatory effect. Some groups suggested that the increased leptin levels in Cushing's syndrome patients are only BMI dependent (41, 235), but others found disease-specific differences, independent of BMI (134, 157, 248). Discrepancies could be due to the fact that in most of the studies, leptin was measured at one single time point, while assessment of leptin secretion at frequent intervals over 24 h showed the existence of a non-BMI-dependent increase (133, 143).

Leptin Regulation of the Hypothalamo–Pituitary–Adrenal Axis

One of the characteristic features of genetically obese mice and rats is the presence of hypercorticosteronemia (79). Since leptin administration corrects the hypercorticosteronemia of *ob*/*ob* mice, it was proposed that leptin could play a major role in the regulation of the HPA axis (2). Subsequent observations supported this conclusion, although the mechanisms involved are not yet clear. The finding that icv administration of CRH reduced food intake (198) and

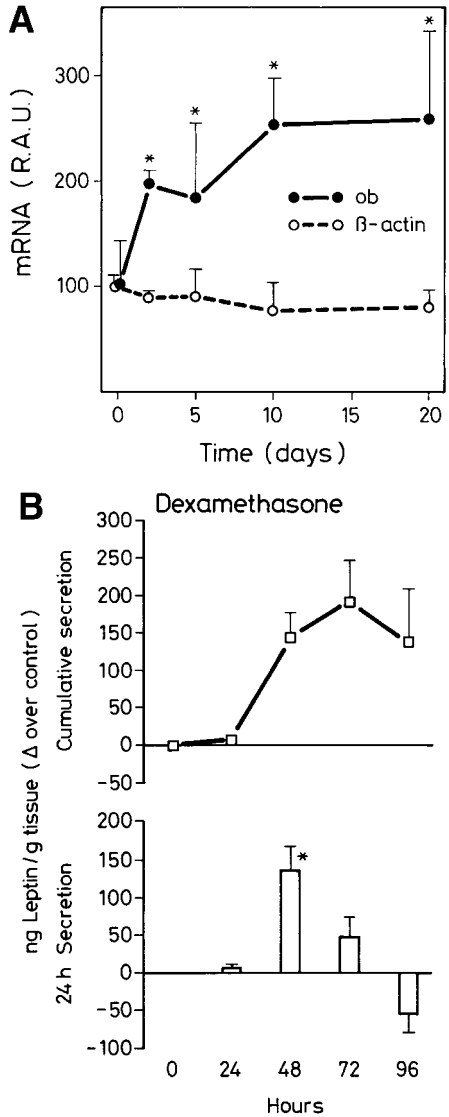


FIG. 11. (A) Kinetics of induction of adipose tissue leptin mRNA after *in vivo* treatment with hydrocortisone (100 µg/g bw per day) in adult male rats. *$p < 0.05$. Redrawn from (59) with permission of the publisher. (B) Stimulatory action of dexamethasone 10^{-7}M on *in vitro* leptin secretion from human omental adipose tissue from female donors. *$p < 0.05$. Redrawn from (30).

increased energy expenditure, while anti-CRH antibodies decreased the anorectic effect of leptin (85), indicates the existence of an interrelationship between these two peptides. Moreover, the presence of abundant leptin receptors in CRH-containing neurons of the parvocellular part of the paraventricular nuclei supports a role for leptin in the regulation of the hypothalamo–pituitary axis (95). However, data regarding the effects of leptin on hypothalamic CRH

synthesis and secretion are somewhat conflicting. Leptin has been reported to increase basal CRH secretion and to inhibit hypoglycemia-induced CRH release from hypothalamic fragments *in vitro* (Fig. 12A) (52, 102). Moreover, discordant results were obtained *in vivo.* Thus, while treatment of *ob*/*ob* mice with leptin for over 5 days did not alter CRH mRNA levels in the paraventricular nuclei, leptin administration to fasted rats, a model of hypoleptinemia, increased CRH mRNA levels in these nuclei (210). Although some workers found a marked induction of c-*fos* in the paraventricular nuclei after icv leptin administration (239), others failed to find a change of c-*fos* in the medial division of the nucleus (66).

The reasons for the discrepancies are at present unclear. In some studies leptin was administered systemically, while in others it was given icv. The paraventricular nucleus of the hypothalamus is divided into several functional regions, which may well be subject to differential regulation. On the other hand, neurons located in the dorsal, ventral, and lateral parvocellular regions give rise to descending inputs to autonomic centers, while the ones that project to the median eminence are concentrated in the medial region. Finally, while some authors assessed CRH or c-*fos* gene expression in specific regions of this nucleus, others assessed the nucleus as a whole.

Nevertheless, a major role of leptin in the regulation of the HPA axis was demonstrated by the findings that leptin reversed both starvation and stress-induced increases in ACTH and/or corticosterone levels (2, 102). Since convincing evidence suggests that these responses are mediated, to a large extent, by an increase in hypothalamic CRH release, the data imply that leptin acts by inhibiting CRH release. Although data on the action of leptin on ACTH are scarce, leptin does not appear to alter ACTH release *in vitro* (102). Taking into account that acute changes in glucocorticoid levels do not seem to affect circulating leptin levels (see above) and that the circadian changes in leptin are preserved in patients with perinatal transection of the pituitary stalk (186), the results indicate that leptin can influence pulsatile ACTH and cortisol release. Whether this effect of leptin on the HPA axis is exerted at a central or a peripheral level is still unclear. Although indirect evidence suggests that leptin acts in the hypothalamus to inhibit CRH release from the parvocellular regions of the median eminence (102), possibly by influencing hypothalamic opioid tone (245), reports directly addressing this possibility are not in agreement.

The possibility that leptin could act directly on the adrenal gland by inhibiting cortisol secretion is supported by the presence of the long form of the leptin receptor in the adrenal cortex (188). Although not a universal finding, it has been reported that leptin inhibits *in vitro* ACTH-stimulated cortisol secretion by rat, human, and bovine adrenal cells (Fig. 12B) (16, 188); this could explain the inverse interrelationship observed in plasma fluctuations among these hormones (Fig. 10). However, it should be noted that the inhibitory effect of leptin on cortisol was observed only after several hours of exposure to leptin, which again argues in favor of centrally mediated effects of leptin on the acute fluctuations of activity of the HPA axis.

These data indicate that glucocorticoids are able to stimulate leptin synthe-

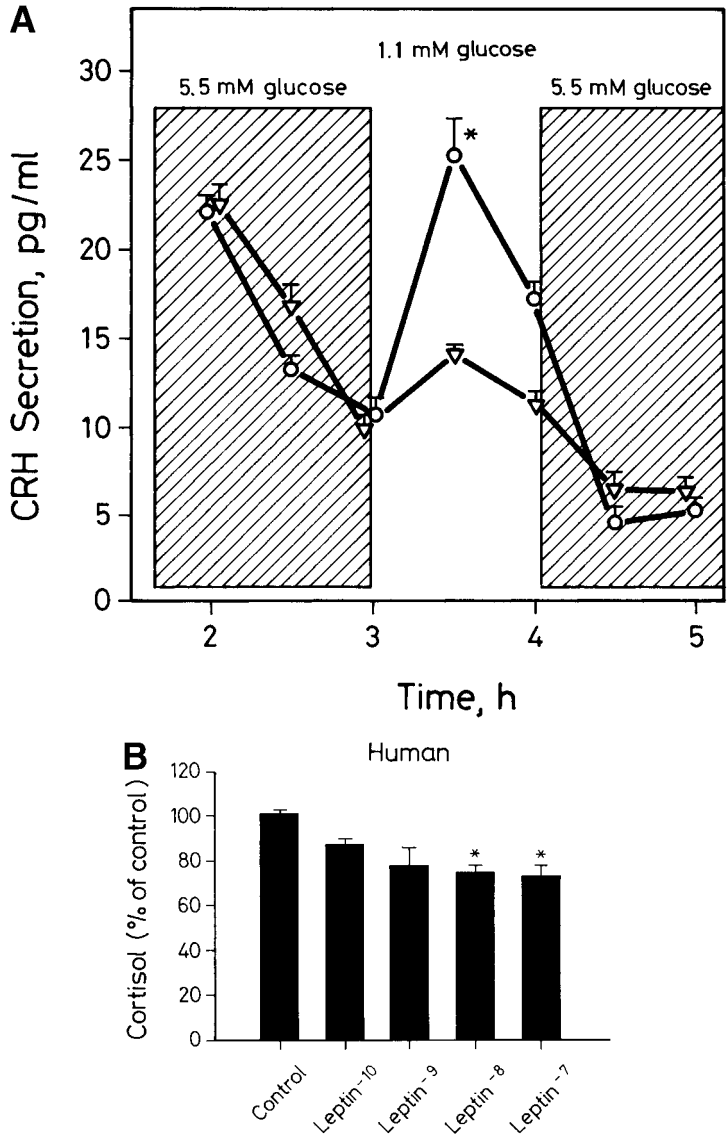


FIG. 12. (A) Inhibition by leptin of hypoglycemia-induced CRH secretion from perifused rat hypothalami. After 3 h in perifusion at 5.5 mM glucose (crossed bars), the medium was changed to 1.1 mM glucose (white bar). Triangles, treatment with leptin; circles, vehicle. $^*p < 0.05$. Redrawn from (102) with permission of the publisher. (B) Effect of 24-h preincubation with serum free-medium (control) or graded concentrations of leptin on ACTH-stimulated cortisol secretion from dispersed human adrenal cells. $^*p < 0.05$. Redrawn from (188) with permission of the publisher.

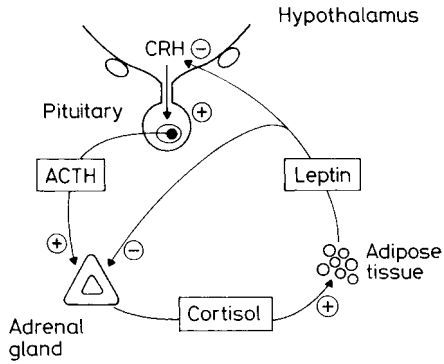


FIG. 13. An integrated view of the interrelationship between leptin and the hypothalamic–pituitary–adrenal axis.

sis and secretion by the adipocytes, whereas rising leptin levels inhibit the activity of the adrenal axis both by inhibiting hypothalamic CRH release and by acting directly on the adrenal gland, suppressing cortisol secretion (Fig. 13). The suppressive effect of leptin on the hypothalamo–pituitary–adrenal axis activity may be of physiological significance, since increased glucocorticoid levels blunt the central effects of leptin on food intake (256). On the contrary, during starvation or stress, decreased leptin secretion could facilitate the responsiveness of this axis, a fact which may be important for survival. Finally, although *ob*/*ob* mice exhibit increased basal secretion of glucocorticoids throughout their lives (63), human subjects with leptin or leptin receptor mutations have ACTH and cortisol levels in the normal range (43, 225). Whether in these patients the glucocorticoid response to stress situations is impaired remains to be established.

LEPTIN AND PROLACTIN

Although only a small number of studies have been conducted on the interaction between prolactin (PRL) and body weight, it has been reported that hyperprolactinemia in humans may be associated with a relatively high rate of obesity, and weight is lost after normalization of serum prolactin levels (92, 129, 173). On the other hand, alterations in the neuroregulation of PRL secretion have been described in an state of high leptin levels such as obesity. Data regarding the interaction of leptin and prolactin are scarce but the fact that in *ob*/*ob* mice leptin partially restores lactation (35) may be relevant. It has been reported that leptin increased PRL secretion *in vitro* albeit only at high concentrations (10^{-7} to 10^{-5} M) (253). On the other hand, leptin antibodies delayed the preovulatory surge of prolactin, while the blunted PRL surge of starved rats was reversed toward normality by icv leptin administration (125), findings that await further evaluation for their physiological meaning.

It is possible that prolactin exerts a stimulatory action on leptin secretion since we have recently found that ovariectomized rats treated with prolactin showed a marked increase in both leptin mRNA and serum leptin levels. This stimulatory effect of PRL was dependent on the metabolic status of the animal since it was not observable in food-deprived rats. Furthermore, this action was observable *in vivo* but not *in vitro,* suggesting that the PRL action was not exerted directly on the adipose tissue but through an indirect mechanism (O. Gualillo, F. Lago, M. Garcia, C. Menendez, R. Señaris, F. F. Casanueva, C. Dieguez, submitted). Future studies are needed with regard to the influence of leptin on *in vivo* PRL secretion, as well as the assessment of leptin levels in patients with hyperprolactinemia, before firm conclusions can be reached on the interaction between leptin and prolactin.

LEPTIN AND PITUITARY TUMORS

Studies regarding leptin receptors in normal human anterior pituitary gland and pituitary tumors have yielded interesting data. As both receptor isoforms are expressed in fetal human pituitary, leptin may well be involved in the regulation of normal pituitary development (216). The long isoform is strongly expressed in 90% of GH, prolactin, and nonfunctioning pituitary tumors (117, 216). Leptin markedly increases *in vitro* GH secretion from fetal anterior pituitary cells at early stages of gestation, and this stimulatory effect disappears at the end of gestation (216). Taken together, these data suggest that leptin can play a major role in anterior pituitary gland development or in embriogenesis (111) and that the expression of the long isoform of the leptin receptor in pituitary tumors may be related to the powerful angiogenic actions reported for leptin (217). An unexpected finding is the synthesis of leptin by pituitary tumors (117).

FUTURE DIRECTIONS

Although the possibility that leptin or a leptin analog might be widely used in the treatment of obesity is unclear at present, the discovery of leptin has greatly advanced our understanding of adiposity and the regulation of energy balance. For the first time, there is a wealth of information about a hormone produced by adipose tissue that is under neuroendocrine control. Furthermore, data gathered in recent years have provided conclusive evidence that leptin serves as a linking signal between nutritional status and neuroendocrine function. This is important in body homeostasis, since the secretion and biological actions of most, if not all, anterior pituitary hormones are markedly dependent on nutritional status.

Despite this progress, many questions about the neuroendocrine role of leptin remain to be answered. Data regarding the effects of leptin on human anterior pituitary hormone secretion in different physiological and pathological

settings are eagerly awaited. The regulation of the different isoforms of leptin receptors in different CNS areas needs to be clarified. Although there are clear data regarding the interaction of leptin with NPY, further studies assessing the anatomical and functional interrelationship with other orexigenic and anorectic peptide pathways need to be systematically assessed. This will hopefully lead to a greater understanding of the regulatory mechanisms involved in pituitary hormone secretion, adaptation to different environmental situations, and pathophysiological mechanisms of different disease states. In summary, data gathered in recent years in leptin physiology have brought together the neuroendocrine mechanisms linking nutritional status and pituitary function. Thus, one of the most elusive areas of research up until now is becoming a rewarding one, with important basic and clinical implications.

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