Leptin and Human Reproduction

Michel L. Aubert, Dominique D. Pierroz, Nadine M. Gruaz-Gumowski,

Béatrice Sudre-Vuagnat, Paula D. Raposinho, Jean-Michel Dubuis,

Pierre Broqua, and François P. Pralong[1]

Division of Biology of Growth and Reproduction, Department of Pediatrics, School of Medicine, 1211 Geneva 14; and Division of Endocrinology and Metabolism, Department of Medicine, University School of Medicine, 1011 Lausanne[1], Switzerland

Corresponding author:

Dr Michel L. Aubert,
Hôpital des Enfants, HUGs,
6, rue Willy-Donzé, 1211 Geneva 14, Switzerland.
Phone (41 22) 382 45 68;
Fax (41 22) 347 59 79;
Email aubert@cmu.unige.ch

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ABSTRACT

The discovery of **leptin,** this hormone originating from adipocytes, has generated an extraordinary interest in the understanding of the relationship between metabolic status and the neuroendocrine system, in particular, the neuroendocrine regulation of reproduction. Following the initial demonstration that leptin administration to leptin-deficient, sterile *ob/ob* mice could activate their reproductive axis, and also that treatment of fasting, normal mice with leptin could "protect" neuroendocrine secretions and prevent the changes that are associated with fasting, the concept has emerged that a normal leptin secretion is a prerequisite for normal reproductive function, leptin acting as a permissive factor. Initiation and maintenance of normal reproductive capacity necessitate adequate metabolic conditions. The time for initiation of puberty has been associated with proper advance in acquisition in body weight or body fat and/or harmonious eating habits. Malnutrition, either inadequate caloric or protein intake, has been associated with delay in puberty or inactivation of the hypothalamo-pituitary gonadal axis. Eating disorders are known to affect gonadal function such as the typical situation of anorexia nervosa, or self-inflicted food restriction, that rapidly results in hypogonadism. Internal cues related to metabolism or nutrition, among them, leptin plasma level, could not only affect the hypothalamic stimulation of the gonadotropic axis, but also modify sexual behavior. Several unfavorable metabolic situations, as they can be studied in animal models, are associated with low plasma leptin, increased secretion of hypothalamic Neuropeptide Y (NPY), and hypogonadism, and a causal relationship has been evoked. Severe dietary restriction in

juvenile female rats is associated with low plasma leptin and sexual immaturity. Plasma leptin levels are very low in untreated patients with anorexia nervosa. Whereas leptin administration has been shown to advance sexual maturation in mice, no such effect has been seen in rats, or in monkeys, and a role of leptin as a signal for the onset of human puberty is still controversial. The targets for leptin action for the control of *feeding* behavior not only include NPY neurons, but also POMC, CART and other neuronal systems that likely to also modulate the reproductive axis. There is a trend for coordinated regulation of ingestive and reproductive behavior, thus a better knowledge of these mechanisms will be very useful for the understanding of such diseases like anorexia nervosa and other reproductive disorders.

Key words：Leptin, Neuropeptide Y, Puberty, CART, Melanocortins

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INTRODUCTION

The concept that the reproductive function highly depends upon nutrition or metabolic conditions related to food intake in general is well accepted both in human medicine and in animal models (1,2). Timing of puberty has been related to nutrition (1,3). In female subjects, it has been well demonstrated that adequate calorie and protein intake are necessary for allowing normal reproductive cycles. This type of requirement was found less stringent in males (1). It has been postulated that time of onset of puberty in girls could be best correlated by the acquisition of a minimum amount of fat stores (3), but the mechanism for transmission of such effect of body fat on the hypothalamic centers that are responsible for the triggering of pubertal release of GnRH pulses has been elusive (2). Furthermore, adverse metabolic conditions such as malnutrition, type-1 diabetes, obesity, or intensive exercise are known to be associated with reduced or abolished reproductive function secondary to reduction of the hypothalamic drive of gonadotropin secretion (1,4,5). The nature of the central inhibitory mechanisms of gonadotropin secretion imposed by unfavorable metabolic conditions is still unclear. Several neuropeptides or neurotransmitters such as GABA (6), POMC gene products such as α-MSH (7), or Neuropeptide Y (8,9) have been implicated as possible regulators of GnRH release. The discovery of **leptin**, an hormone secreted by the adipose tissue (10) that clearly acts at the hypothalamic level, has brought a lot of new interesting concepts in this relationship between acquisition of fat stores, metabolic status, and various neuroendocrine functions, in particular, the reproductive endocrine axis.

LEPTIN AND REPRODUCTION

The characterization of the product of a gene that is defective in the obese *ob/ob* mouse, the so-called leptin, has generated an extraordinary interest for the relationship between metabolic status, reproductive function, and the neuroendocrine role of NPY (10,11). The leptin gene is mutated in the *ob/ob* mouse and the absence of the gene product is responsible for obesity in this animal, since treatment with leptin obtained by recombinant technology rapidly reduces food intake and body weight (10). Although it was recently shown that leptin is synthesized in the placenta (12) and the stomach (13), its main place of synthesis is the white adipose tissue. Leptin is secreted into the general circulation and modulates food intake, most likely by an action at a central level (14). The concept quickly emerged that leptin controls food intake at

least in part through an action on NPY release (14-17), thus bringing the missing link for the understanding of the regulation of NPY release in function of prevailing metabolic conditions.

A great lot of interest has focused on the role of leptin in the control of feeding and the physiopathology of obesity (18). Furthermore, since NPY has been clearly implicated as a regulator of neuroendocrine functions, one of them being the gonadotropic axis, the indication that leptin could regulate reproductive function possibly by an action mediated by NPY, received immediate attention when treatment of obese *ob/ob* mice with leptin was shown not only to correct their excessive food intake and body weight gain, but also to restore fertility (19,20) through an activation of the GnRH-LH-gonadal axis (21). The description of coexpression of leptin receptor and preproneuropeptide Y mRNA in the arcuate nucleus of mouse hypothalamus (16) have suggested that leptin action on the reproductive axis could be mediated at least in part through an action on NPY neurons. It should be stressed however that the pattern of leptin receptor expression within the hypothalamus supports the possibility of direct leptin control of GnRH secretion as high concentration of leptin receptor are present in the ventromedial and arcuate nuclei which contain a significant number of GnRH neurons (22-25). This possibility is also supported by the expression of leptin receptors on GnRH neurons maintained *in vitro* (26).

Evidence that hypogonadism is associated with decreased leptin secretion and increased hypothalamic NPY activity has been suggested in the case of fasting (27), or delayed puberty (28-30). Leptin has been shown as well to directly affect pulsatile secretion of LH in rats by preventing the LH release during fasting (31), or indirectly by increasing metabolic fuel oxidation (32). Humans with mutations in the leptin gene or leptin-receptor gene are infertile (33,34). It is expected that leptin secretion would be low or suppressed in type-1 IDDM diabetes, since *ob* gene expression in rat adipose tissue is decreased in this situation (35). Indeed, leptin secretion is low in Streptozotocin-induced type-1 diabetes (36-37) and reciprocally, gene expression for NPY is elevated (37,38). Leptin has been shown as well to control growth hormone secretion in the rat, an action mediated by the NPY neuronal system (39-40).

Whereas the role of leptin, modifying the secretion of hypothalamic releasing hormones, in particular GnRH in the case of reproduction, is now well established, putative roles of leptin at pituitary or gonadal levels have been described but the physiological meaning of the available observations is still difficult to conceptualize. Leptin has been shown to increase LH and FSH release from isolated pituitaries *in vitro* (41). Functional leptin receptor have been found in pituitary glands at different ages and conditions (42-43). Direct inhibitory effects of leptin on cultured granulosa cells from rat and bovine have been described (44,45), and full length leptin receptor have been described in the human ovary (46). Functional roles for leptin in follicle physiology (47,48), and testicular function (49,50) have been suggested. Whether direct effects of leptin at these targets are seen normally in vivo, and what these effects would be, are unknown at present. It can nevertheless be postulated that regulation of ovarian (testicular) function by circulating leptin could arise not from minute to minute changes in plasma levels, but from the permanent changes in concentration range related to alterations in metabolic conditions, with, for example, a modification of gonadal function resulting from continuously low circulating leptin.

LEPTIN AND THE CONTROL OF SEXUAL MATURATION

It has been hypothesized for a long time that onset on puberty in adolescents would correlate with acquisition of body mass, fat mass, or other metabolically related factors, that would tell the brain that the body is sufficiently developed to afford the pubertal changes and/or onset of reproductive life depending the species (3). This hypothesis implied that a humoral factor would trigger the pubertal increase in gonadotropin secretion, thus the existence of leptin had been anticipated already in 1980. It is known that sexual maturation is delayed when metabolic conditions are not satisfactory, as in food or protein restriction (51, 52). We have shown that central NPY infusion permanently delays sexual maturation (30), and we suggested that NPY could at least in part be responsible for this delay in sexual maturation at least in the rat (52). The possibility that leptin also controls sexual maturation/function by the same axis has been evoked (29,53). Very recently, evidence has been provided that NPY could also act as an hypothalamic brake restraining the onset of of puberty in primates (54).

a) Leptin secretion and sexual maturation

Plasma leptin was investigated by radioimmunoassay starting at 24 days of life (d) in normal female rats, and in rats subjected to food restriction. Plasma leptin levels were low at 24 d (289$\pm$65 pg/ml) compared to adult levels at 59 d (957$\pm$73 pg/ml). Plasma leptin then steadily increased during the juvenile period, and reached 740$\pm$56 pg/ml at 41 d at time of vaginal opening (VO). At this age (41 d), food-restricted rats had no VO and low plasma leptin (19-360 pg/ml). In rats eating 60% of normal food allowance, spontaneous VO was observed between 55-60 d, representing a delay of approximately 20 days, and plasma leptin was 740$\pm$48 at 59 d. With a daily food intake reduced to 7-8 g/d (representing 36% of normal food intake), VO and any kind of reproductive activity were permanently prevented, and plasma leptin concentration was very low. Following switch to ad-libitum feeding at 53 d, plasma leptin immediately increased and reached normal levels after 2 days. VO occurred 4 days later (51, 55). Thus, circulating leptin could represent a signal to the hypothalamus, indicating that nutritional input is again or finally compatible with onset of sexual function. In order to better demonstrate that leptin could represent a permissive factor for the timing of sexual maturation, food-restricted rats received an infusion of mouse leptin (10 μg/d, Lilly) into the lateral ventricle starting at 53 days of life. Such infusion resulted in a small decrease in body weight as compared to food-restricted rats not receiving leptin (91$\pm$1 to 80$\pm$1 g), indicating increased energy expenditure as seen in Figure 1. Vaginal opening occurred in 8 out of 9 rats receiving leptin despite the situation of extreme malnutrition (55). Thus leptin clearly can act on the GnRH neuronal system to bring a "metabolic signal", in this case an inappropriate signal, that puberty can take place.

b) Leptin and the timing of sexual maturation

Two groups have demonstrated independently that leptin administration could *advance* sexual maturation in mice (29,53), and there has been a tendency to extrapolate this finding to other mammalian species. In the normally fed rat, leptin administration did not *advance* sexual maturation, but partially prevented the negative effects of food restriction induced by leptin on the timing of sexual maturation (29). Infused either centrally, or subcutaneously, we found that leptin administration induced a marked, dose-dependent reduction in food intake, resulting in

an important reduction of body weight gain. Sexual maturation of pair-fed rats, subjected to a food restriction that matched the restriction induced by leptin, was clearly delayed. Leptin treatment only partially rescued sexual maturation, in our study, 4 rats out of 9 (56,57), confirming Cheung et al (29) study. There is therefore a clear difference between the mouse and the rat for this effect of leptin on sexual maturation: in the mouse leptin administration advanced sexual maturation (29,53), a finding that could not be reproduced in the rat (56,57). In a monkey study, Plant and Durrant (58) could not demonstrate any correlation between the normally elevated plasma leptin levels and onset of pubertal increases in LH and testosterone during puberty in the male monkey. Clearly, a normally elevated secretion of leptin is necessary for onset of pubertal secretion of gonadotropin, but a sudden rise in leptin secretion does not represent the signal for such increase. The same phenomenon is true for human puberty. The work of Mantzoros et al (59) suggests that leptin secretion peaks shortly before puberty in boys. It is our impression that again, like in the monkey, leptin represents a permissive factor for the onset of human sexual maturation, not a signal (51,60). Very recently, transgenic mice overexpressing leptin have been developed and shown to have increased glucose metabolism and insulin sensitivity, with as a result, a complete disappearance of white and brown adipose tissue, thus developing a phenotype of "skinny" mice (61). Interestingly, these mice have accelerated puberty, perfectly in line with the concept that leptin is promoting early pubertal development in mice (62). The counterpart of this observation is that a late onset of hypothalamic hypogonadism was present in these skinny mice that is highly compatible with the extreme metabolic situation of these female rats, and reminiscent of some aspects of the syndrome of hypogonadism in hyperactive women (62). In conclusion, plasma leptin concentration is low after weaning in the female rat and progressively increases during the pre- and pubertal periods. In different forms of delayed sexual maturation induced by food restriction, plasma leptin levels remained very low. This is the case for anorexia nervosa patients with amenorrhea (60). It is likely that the rising plasma levels of leptin in the prepubertal period represents a permissive factor indicating that the young animal is metabolically ready to go through the process of sexual maturation. Therefore our data suggest, at least in the rodent, that leptin could be identified as the long sought metabolic signal responsible for the triggering of the onset of puberty in function of metabolic status.

MEDIATION OF LEPTIN ACTION ON REPRODUCTION

Although leptin clearly can influence the reproductive axis positively, the question remains to understand how leptin acts on presumably the GnRH neuronal system, either by a direct action, or through a mediation by brain neuropeptides (2,63). The simplest possibility would be that the fat-derived hormone regulates GnRH activity directly. This is questionable because it is difficult experimentally to change levels of leptin without altering many other nutritional factors which could be involved in the modulation of reproduction as well. Many data support the notion that the reproductive actions of leptin involve actions in the brain and more specifically in the hypothalamus. The long form of the leptin receptor that is responsible for signal transduction is heavily expressed in the arcuate nucleus and ventromedial hypothalamus (64), areas important for controlling GnRH release and sexual behavior, respectively. Leptin stimulated GnRH release from isolated hypothalamic explants in vitro (41) and ICV administration of leptin antibodies reduced pulsatile LH release (65). Also, ICV administration of leptin at doses that did not influence peripheral leptin concentrations restored LH secretion during fasting in rats (55).

Taken together, these data suggest that leptin acts centrally to influence reproduction, but do not answer the question of whether these actions are exerted directly upon GnRH neurons or indirectly through interneural inputs. To date, leptin receptors have not been identified on GnRH neurons, favoring the idea of neuronal intermediaries of the actions of leptin on GnRH release. The identity of neural systems that may link leptin and GnRH release is unknown, but recent data from NPY knockout mice (66) and MC4 receptor knockout mice (67), both of which are fertile, would indicate that these two systems are not essential for mediating the reproductive effects of leptin. Nevertheless, interpretation of data with transgenic mice should be done with caution since alterations in the brain circuitry may have happen during transgenesis and mechanisms that are valid in wild type animals may have been invalidated in transgenic models.

a) The Neuropeptide Y (NPY) angle

Neuropeptide Y (NPY) that is tighty regulated by leptin, has been shown to be clearly implicated in the regulation of gonadotropin secretion in several species (8). NPY is synthesized in the arcuate nucleus of the hypothalamus and many other locations in the CNS, and appears to control metabolic functions such as food intake and thermogenesis, and also reproductive parameters such as sexual behavior or gonadotropin secretion (for review see Kalra, 8, and Pierroz et al, 9). Intracerebroventricular (icv) injection of NPY has been shown to stimulate LH secretion in steroid-intact rats, and to inhibit such secretion in castrated rats (8). Five receptor subtypes for NPY have been identified for the transduction of the NPY action (68). NPY gene expression is increased in diabetic rats (37,38,69), and in many other metabolically adverse situations (for review, see Gruaz et al ,52). We have shown that central infusion of NPY into the lateral ventricle to normal, intact rats very rapidly inhibits the gonadotropic axis, leading to arrest of estrous cyclicity in female rats (70), and major decreases in weight of seminal vesicle, testis and prostate in male rats in the face of very low testosterone secretion (9). We have further demonstrated that central NPY infusion completely inhibits the pulsatile secretion of LH, stressing the fact that NPY acts centrally for producing hypogonadism (71). We have postulated that impaired reproductive function in adverse metabolic conditions such as fasting or diabetes could be due to excessive hypothalamic NPY release (52). We recently demonstrated that the NPY receptor subtype Y5 is involved in the transduction of this inhibitory action of NPY in the rat (72). Conversely, evidence for a stimulating pathway involving the Y4 receptor has been provided (73,74). The inversed relationship between leptin action and hypothalamic synthesis has been highlighted by Ahima et al (27) in their elegant study demonstrating that leptin administration prevents the fasting-induced neuroendocrine changes. They showed that leptin administration maintained a normal LH, TSH and ACTH secretion despite fasting by concomitantly preventing the rise in hypothalamic gene expression for NPY, that is commonly seen during fasting. The demonstration of this leptin-NPY axis may not be exclusively a rodent story, since very recently Plant and colleagues offered a demonstration that NPY may act as well as a hypothalamic brake restraining the onset of puberty in their model of male Rhesus monkeys (54).

b) The Melanocortin (MC) angle

Among other regulators of ingestive behavior that are regulated by leptin, Neuropeptide Y (NPY) is known to represent the most powerful orexigenic factor, and α -MSH, a melanocortin

(MC) peptide, to induce satiety through the MC4 receptor subtype (75). Interactions between these two systems in relation to leptin tone have been evoked (67,75). Disruption of the MC4 receptor system, either by null mutation (67), or by constant antagonism of this receptor subtype (75-77) results in hyperphagia, maturity-onset obesity, hyperinsulinemia and hyperglycemia (75). Aberrations in pigmentation have been also described. The question remains whether activation of the MC4 receptor subtype may influence the reproductive axis and possibly transduce a leptin message into modifications of GnRH release. The genetic models of disruption of this receptor subtype have yielded animals that are fertile, thus indicating that this receptor is not essential for leptin action.

Using a 7-day infusion into the lateral ventricle of male rats with either the MC3/4 receptor antagonist SHU9119, or porcine NPY (10 nmol/d) we were able to produce in both cases an important obesity syndrome. As expected, icv infusion of NPY produced a profound hypogonadism, whereas, the obesity induced by permanent blockade of the MC4 receptor subtype was not associated with any form of decreased reproductive function (78). Thus, in the first analysis the melanocortin signaling system, that is regulated by leptin, does not appear to control reproduction. This concept is consistent with recently published data by Hohmann et al (79) who addressed this question by using the otherwise sterile ob/ob mouse that becomes fertile upon leptin treatment (19,20). They administered the MC antagonist SHU9119 together with leptin to ob/ob mice and could demonstrate the same process of initiation of sexual function as with leptin alone, whereas leptin's effects on feeding and body weight gain were attenuated (79). Taken together these observations make unlikely that α-MSH and the MC receptor system play a significant role in the transduction of leptin action on the gonadotropic axis.

c) The Cocaine and Amphetamine-Regulated Transcript (CART) angle

Cocaine and Amphetamine-Regulated Transcript (CART), a brain-located peptide, represents a satiety factor closely associated with the action of leptin and NPY (80). Food-deprived animals show a pronounced decrease in expression of CART mRNA in the arcuate nucleus. In animal models of obesity with disrupted leptin signalling such as *ob/ob* mice, CART mRNA is almost absent from the arcuate nucleus. Peripheral administration of leptin to obese *ob/ob* mice stimulates CART mRNA expression. An antiserum against CART increases feeding in normal rats, indicating that CART may be an endogenous inhibitor of food intake. When injected icv into rats, recombinant CART peptide inhibits both normal and starvation-induced feeding, and completely blocks the feeding response induced by NPY (80). Since the axis leptin-NPY is also involved in some aspects of regulation of the gonadotropic axis, and CART counteracts the effects of NPY in general, it could be suspected that a leptin-CART axis, able to modify GnRH/LH release, could exist.

Lebrethon and coworkers, using an *in vitro* model, have shown that NPY and leptin can stimulate the GnRH pulse generator of prepubertal rats through distinct mechanisms, and they suggested that the Y5 receptor subtype is involved in the NPY driven stimulation (81). These findings suggest that leptin is not activating GnRH directly but that a neuromodulator is needed (81). In a more recent study they showed that CART is able to mediate the stimulatory effect of leptin on the rat GnRH pulse generator in this *in vitro* model (82). They showed that an anti-CART serum prevents the stimulatotry action of leptin on the GnRH pulse generator and

that CART is able to reduce GnRH interpulse interval which represent an activation in this system (82). They further showed that the hypothalamic GnRH pulse generator of Zucker (*fa/fa*) rats that are insensitive to leptin, can be directly activated by CART. These data strongly suggest that CART could be one mediator of the leptin stimulatory action on the gonadotropic axis. Clearly, such results obtained in an *in vitro* model should be reproduced *in vivo*. If CART would be active *in vivo*, it would represent the second neuromodulator after NPY primarily involved in the regulation of feeding but also endeavored to regulate the gonadotropric axis.

The Glucose angle

The view that leptin may act in concert with other metabolic signals has been evoked. Leptin may regulate insulin-stimulated glucose uptake, a key factor regulating glucose availability. Glucose availability may well be an authentic metabolic signal with the idea that variation in glucose availability could provide proper input for the control of GnRH secretion. Studies in the Syrian hamster have set the stage for such concept with the demonstration that oxidizable metabolic fuels, such as glucose, may be important in regulating estrous cyclicity (83). In both sheep (84) and rat (85), there is strong evidence that glucose availability may be a regulator of LH secretion. Combined administration of 2-deoxyglucose (2DG), a competitive inhibitor of glucose utilization, and methyl palmoxirate, an inhibitor of fatty acid oxidation, at doses which when delivered separately are without effect, disrupts estrous cyclicity in hamsters (86). Administration of higher doses of 2DG alone reduces pulsatile GnRH release in sheep (87), pulsatile LH release in rats (85) and decreases C-fos expression in hamster GnRH neurons (88). Not unexpectedly then, normal GnRH neuronal activity is dependent upon sufficient energy availability.

Other evidence has become available to suggest that leptin action may work through changes in glucose availability. A recent study from Schneider’s group on the Syrian hamster (39) reveals that the positive influence of leptin on estrous cyclicity during fasting is inhibited by the competitive glucose antagonist (2DG). This finding raises the possibility that the underlying mechanism for leptin’s ability to restore reproductive activity during fasting is due to its ability to improve glucose availability. Perhaps leptin acts centrally at a glucosensor to stimulate glucose uptake. Leptin could act centrally as a glucosensor to stimulate glucose uptake. It would be interesting that that such regulation by leptin occurred in the area postrema, a hindbrain circumventricular organ that is thought to serve as an important glucose detector for the regulation of GnRH secretion (89). Additional study will be necessary to clarify actions of leptin in the periphery and to define the relative importance of these actions in regulating reproduction. Probably studies with leptin-deprived ob/ob mice would be appropriate.

CONCLUSIONS

Inappropriate metabolic conditions often result in hypothalamic hypogonadism, both in rodent models and in higher species including the human species. The adverse metabolic conditions lead to suppression of the GnRH drive on gonadotrophin secretion. Only the threshold for such suppressive effect varies from species to species, or according to age or gender. The mechanisms of suppression of pulsatile release of GnRH are still not well understood and many hypothalamic neuromodulators have been evoked as potential central inhibitor of GnRH release. We postulated that NPY could play a key role for this inhibition, since gene expression

for NPY is increased in most forms of "metabolic hypogonadism" in the rat, and we demonstrated that exogenous NPY could reproduce these forms of hypogonadism (52). The discovery of leptin, its role on metabolic parameters such as food intake, the role of NPY, and probably CART as possible neurotransmitters of the hypothalamic action of leptin, finally, the striking observations that leptin tone could control the maintenance of reproductive function clearly revolutionized the concepts prevailing for the metabolic control of reproductive function and strongly suggested a role of leptin to act either directly on GnRH neurons to control reproductive function, or indirectly via a NPY or CART neurotransmission.

Like for the control of feeding, leptin may act on several targets to control reproduction. In a recent study, Xu et al (90) analyzed the 24-h changes in gene expression for NPY, POMC, and Galanin at the hypothalamic level in fed and food-restricted rats, and compared these changes with daily variations of leptin secretion and gene expression for leptin receptor in the hypothalamus. The correlation between changes in leptin secretion and gene expression for NPY or POMC is only partial, indicating that plasma leptin response is mainly driven by meals, and that NPY and POMC gene expression follows an independent pattern. In the food-restricted rats, gene expression for NPY is constantly elevated, and that for POMC remains low over 24-h, demonstrating that no satiety signal is driven any longer by POMC transcripts. Possibly, reduced GnRH secretion in these conditions could result from this decrease in POMC products. Much more work is clearly necessary to fully understand these mechanisms.

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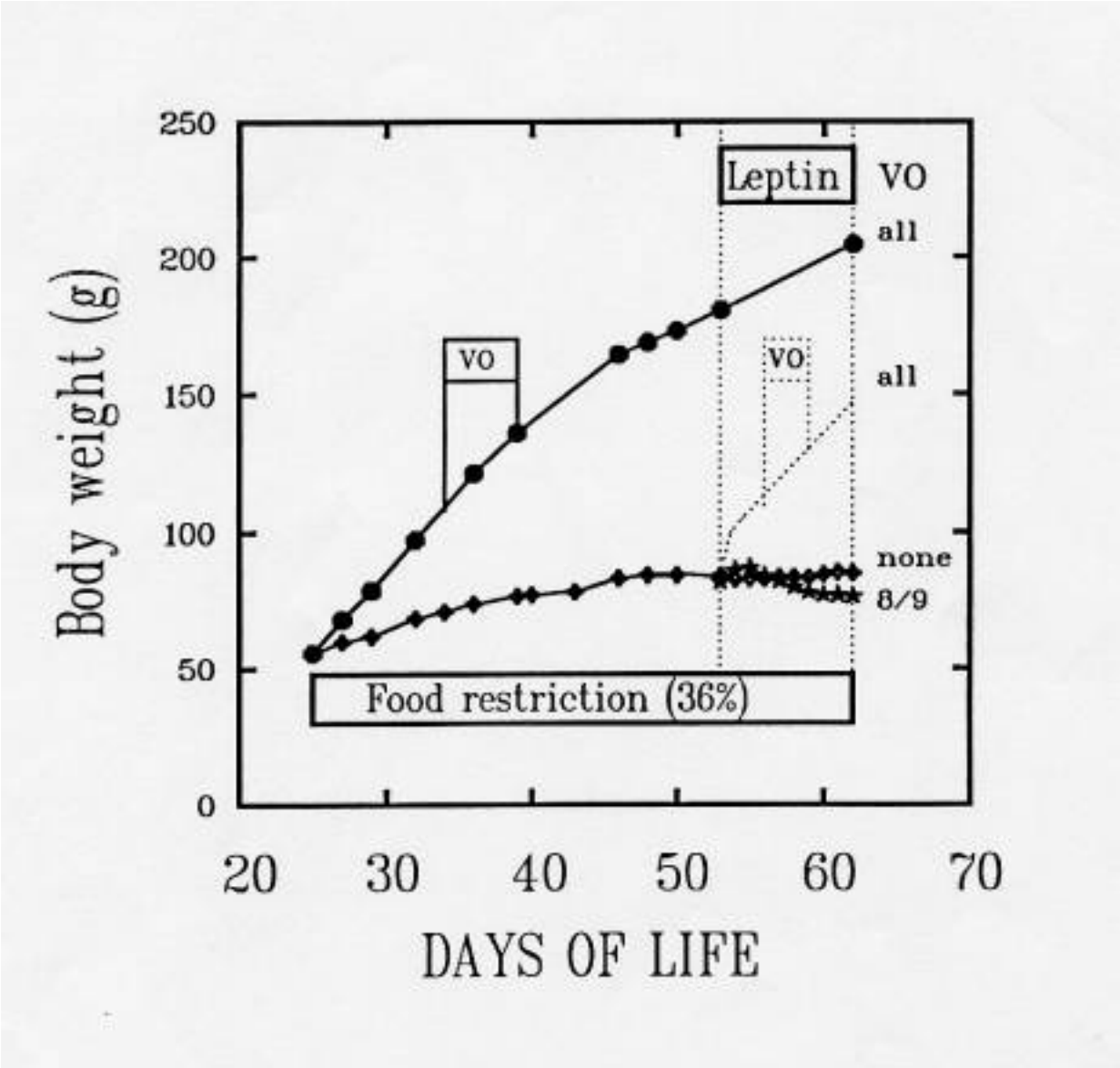

Fig. 1 Model of severe food restriction during sexual maturation in the female rat. Effects of central leptin infusion on body weight gain and vaginal opening (VO) in a paradigm of severe food restriction that prevents sexual maturation. An icv cannula was implanted to food-restricted rats (36% of amount eaten by controls) at days 48-49, and continuous infusion of mouse leptin (star) or vehicle (diamond) was started at day 53. Leptin (Lilly) was administered subcutaneously by mean of Alzet 2001 pumps. Control rats without infusion (circle). Animals were observed until day 62. Two leptin-infused rats experienced VO at day 63, one day after implantation of a second pump. The dotted line represents the data of food-restricted rats that were switched to *ad libitum* feeding at day 53, not receiving a central infusion of leptin (from Gruaz-Gumowski et al, 55).

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