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Regulatory roles of leptin in reproduction and metabolism: A comparative review

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Abstract

Leptin plays an important role in signaling nutritional status to the central reproductive axis of mammals and appears to be at least a permissive factor in the initiation of puberty. The expression and secretion of leptin are correlated with body fat mass and are acutely affected by changes in feed intake. Moreover, circulating leptin increases during pubertal development in rodents, human females and heifers. Effects of leptin are mediated mainly via receptor activation of the JAK-STAT pathway; however, activation of alternative pathways, such as MAP kinase, has also been reported. Although the leptin receptor (LR) has not been found on GnRH neurons, leptin stimulates the release of GnRH from rat and porcine hypothalamic explants. Moreover, leptin increases the release of LH in rats and from adenohypophyseal explants and/or cells from full-fed rats and pigs. In contrast, stimulation of the hypothalamic–gonadotropic axis by leptin in cattle and sheep is observed predominantly in animals and tissues pre-exposed to profound negative energy balance. For example, leptin prevents fasting-mediated reductions in the frequency of LH pulses in peripubertal heifers, augments the magnitude of LH and GnRH pulses in fasted cows, and enhances basal secretion of LH in vivo and from adenohypophyseal explants of fasted cows. However, leptin is incapable of accelerating the frequency of LH pulses in prepubertal heifers, regardless of nutrient status, and has no effect on the secretion of GnRH and LH in full-fed cattle or hypothalamic/hypophyseal explants derived thereof. Similar to results obtained with LH, basal secretion

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of GH from anterior pituitary explants of fasted, but not normal-fed cows, was potentiated acutely by low, but not high, doses of leptin. Mechanisms through which undernutrition hypersensitize the hypothalamic–gonadotropic axis to leptin may involve up-regulation of the LR. However, an increase in LR mRNA expression is not a requisite feature of heightened adenohypophyseal responses in fasted cattle. To date, leptin has not been successful for inducing puberty in ruminants. Future therapeutic uses for recombinant leptin that exploit states of nutritional hypersensitization, and identification of genetic markers for genotypic variation in leptin resistance, are currently under investigation.

Keywords: Leptin; Hypothalamus; Adenohypophysis; Reproduction; Cattle

1. Introduction

The hypothesis for the existence of a peripheral factor that informs the brain of energy status was first proposed in the 1950s [1,2]. This hypothesis formed the basis for further investigations that led eventually to characterization of the obese (o*b*/*ob*) mouse, a homozygous mutant that lacks a critical factor for regulation of body weight. Coleman characterized the phenotype of the obese mouse which included massive obesity, hyperphagia, insulin resistance, and cold intolerance [3]. However, it was not until 1994 that Zhang et al. characterized, through positional cloning and sequencing, the genotype responsible for the syndrome leading to the discovery of leptin [4]. The term *leptin* was taken from the Greek “leptos”, meaning “thin”, and is descriptive of the hormone’s body weight-reducing effects. However, leptin not only normalized the obese, diabetic state in *ob/ob* mice, it also restored fertility. Hence, a concerted effort to characterize the physiological role of leptin and the mechanisms that govern its actions followed, including considerations of its potential for treating obesity in humans, as a hormonal signal to the central reproductive axis, and as a trigger for puberty. The purpose of this review is to summarize on a comparative basis recent developments in the biology of leptin, with a particular emphasis on the role of leptin in regulating reproduction and metabolism in ruminant species important to animal agriculture.

2. Overview of the leptin gene and LR

The leptin gene is highly conserved across species, and is located on chromosome 7q31.3 in humans [5] and on chromosome 4q32 in the bovine [6]. Its DNA sequence has more than 15,000 base pairs and contains three exons, which are separated by two introns [5]. The mouse protein exhibits 83% homology with human leptin [4], and both share many structural similarities to other members of the helical cytokine family, including interleukin-6 (IL-6) and growth hormone (GH) [7,8]. Although adipose tissue is the primary source of leptin, its production has also been observed in a variety of other tissues, including the stomach [9], skeletal muscle [10], fetal cartilage [11], pituitary [12], mammary tissue [13], and placenta [14]. Leptin may be found in the circulation in the free form or complexed with leptin-

binding proteins, and this characteristic appears to be species-specific and dependent upon physiological status [15,16]. In humans, the half-life of free leptin is about 30 min [17], with the kidneys being responsible for approximately 80% of leptin clearance from the peripheral circulation [18]. In addition, leptin secretion follows a circadian rhythm [19], with a nadir early in the morning (08:00–09:00 h), an increase during the day, and a peak between 24:00 and 02:00 h. A circadian rhythm for leptin secretion has not been observed in ruminants [20].

The LR has a single membrane-spanning domain and exists in different isoforms (Ob-Ra, Ob-Rb, OB-Rc, Ob-Rd, Ob-Re and Ob-Rf) that derive from alternative splicing of mRNA [21]. All isoforms have similar ligand-binding domains but differ at the C-terminus, intracellular domain. The Ob-Rb, which contains a long intracellular domain, is the only isoform with both of the protein motifs necessary for activation of the Janus kinase 2 and signal transducers and activators of transcription (JAK-STAT) pathway [22]. Although the JAK2/STAT3 pathway has been considered the major signaling mechanism activated by the LR, mitogen-activated protein kinase (MAPK) [23] and phosphatidylinositol-3 kinase (PI-3K) [23] have also been implicated in LR signaling.

Although originally referred to as the "anti-obesity" hormone, leptin's effects are counteracted in some humans by a natural resistance that is associated with hyperleptinemia. It appears that an intracellular protein induced by LR activation, the suppressor of cytokine signaling-3 (SOCS-3), may mediate leptin resistance at the molecular level within the brain [24], as it effectively blocks leptin signaling. Similarly, the soluble LR (Ob-Re), a potential product of proteolytic cleavage of membrane-bound isoforms in the human [25], can bind leptin in the circulation, augment its half-life, and perhaps contribute to leptin resistance. Leptin has also been linked to regulatory processes as divergent as angiogenesis, hematopoiesis, bone formation, the immune response, diabetes, and general fertility.

3. Neuroendocrine effects of leptin

In mammals, hypothalamic control of gonadotropin secretion is mediated by gonadotropin-releasing hormone (GnRH). The hypothesis that leptin plays an important role in regulating GnRH secretion, and ultimately in reproduction, stems from several findings. First, the *ob*/*ob* mouse, lacking a functional leptin gene, is infertile and has atrophic reproductive organs [26]. Gonadotropin secretion is impaired and the central reproductive axis is very sensitive to negative feedback by gonadal steroids [27,28]. Treatment with leptin rejuvenates the reproductive system in *ob*/*ob* mice, leading to growth and function of the reproductive organs and fertility [26] via secretion of gonadotropins [26,29]. Studies in vitro with hemipituitaries and mediobasal hypothalamic explants of rats have demonstrated that leptin can act directly in both the hypothalamus and the pituitary to stimulate the release of GnRH and LH, respectively [30–32]. Recent studies in our laboratory with cattle support these assertions. However, both sexual maturation and nutritional status are important determinants of how leptin affects the hypothalamic–hypophyseal axis in ruminants [33–35].

3.1. Role of leptin in regulating gonadotropin secretion in cattle and sheep: effects of nutritional status

Ruminants are less acutely responsive to short-term changes in dietary energy intake than monogastrics. However, the peripubertal heifer [36] and estradiol-implanted wether [37] represent two ruminant models in which acute fasting has been demonstrated to restrain the frequency of LH pulses and lower mean circulating concentrations of LH. Moreover, chronic feed restriction reduces the LH pulse frequency in lambs [38,39] and circulating concentrations of LH in ewes [40]. Treatment with leptin does not affect LH secretion in adequately nourished ovariectomized ewes [41] and cows [33] but clearly prevents a reduction in the frequency of LH pulses in fasted, estrogen-treated wethers [37] and fasted prepubertal, intact heifers [42]. Moreover, in early reports, leptin appeared to stimulate secretion of LH in chronically food-restricted, ovariectomized ewes [43]. However, more recent studies from the same laboratory have suggested that only fasted ewes previously fed a normal diet will respond acutely to leptin, whereas long-term nutrient-restricted ewes that were fasted do not respond with an increase in LH secretion [44]. Nonetheless, it is clear that the stimulatory effect of leptin on LH secretion in ruminants is confined predominantly to periods of nutritional stress ([36,37,42,43]; Table 1). In the mature cow, although short-term (2–3 d) fasting is incapable of restraining the frequency of LH pulses, even in moderately thin animals [33,34], leptin stimulated a robust increase in baseline and over-

Table 1
Summary of the effects of recombinant oleptin on the hypothalamic–gonadotropic axis in cattle, including probable site(s) of action

Animal/tissue model	Response	Site of action	Reference(s)
Fasted cow	Increased baseline and mean circulating LH; increase pulse size	Adenohypophysis	[33,34]
Adenohypophyseal explants; fasted cows	Increase basal release of LH	Gonadotrope	[35]
Adenohypophyseal explants; full-fed cows	Increase GnRH-mediated release of LH	Gonadotrope	[35]
Fasted, peripubertal heifers	Prevented fasting-mediated reduction in frequency of LH pulses and increased frequency relative to start of fasting	Hypothalamus	[42]
	Increased GnRH-mediated release of LH	Adenohypophysis	[42]
Fasted cows	Increased plasma LH and IIIV csf GnRH; increased amplitude of LH pulses and size (auc) of GnRH pulses	Hypothalamus/ adenohypophysis	[45]
Adenohypophyseal explants and primary cell cultures, full-fed bulls, steers	No effect	N/A	[47]
Prepubertal, normal- or moderately growth-restricted, prepubertal heifers	No effect	N/A	[86,45]

Abbreviations: auc, area under the curve; csf, cerebrospinal fluid; oleptin, recombinant oleptin; IIIV, third ventricle.

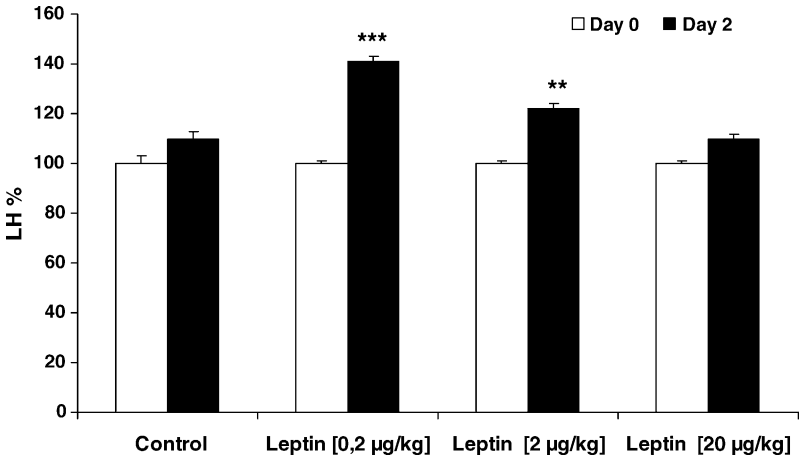

Fig. 1. Dose-related effect of intravenously administered recombinant oleptin on mean concentrations of circulating LH in mature ovarectomized cows. Mean concentrations of LH were greater than controls at the lowest (141% of control; $P < 0.001$) and middle (122% of control; $P < 0.01$) doses employed. Results are expressed as mean percent (±S.E.M.) of the time 0 value on day 0. (Adapted with permission from [34].)

all mean concentrations of LH. This occurs as a result of an augmentation of the size of individual LH pulses [33,34]. These findings, coupled with our recent observations using perifused anterior pituitary explants, are consistent with the view that the effects of leptin on LH secretion in the sexually mature, ruminant female reside to a large degree at the level of adenohypophysis [35]. However, in our most recent studies, we measured GnRH directly in CSF collected from the third ventricle (IIIV) and observed the ability of leptin to increase the concentration of GnRH and size of individual pulses of GnRH. Therefore, it is now clear that leptin-mediated increases in LH secretion in the cow can be effected at both hypothalamic and anterior pituitary levels (Table 1). The inability of leptin to stimulate an increase in circulating concentrations of LH in full-fed cattle and sheep [41,42,46], and in primary cell cultures or explants from full-fed cattle [47], is not completely understood; however, the published literature increasingly supports a consensus that leptin stimulates the hypothalamic–adenohypophyseal axis mainly in nutritionally stressed animals. Additionally, the effects of leptin on the hypothalamic–pituitary axis appear to be exquisitely dose-dependent. Intravenously injected recombinant oleptin causes an inverse, dose-related increase in basal plasma concentrations of LH in ovariectomized, estradiol-implanted cows fasted for 60 h [34]. A dose of 0.2 μg/kg maximized the increase in LH, whereas doses of 2 and 20 μg/kg caused lower and no responses, respectively (Fig. 1). Therefore, the duration and/or amount of exposure to the hormone appear to determine the level of resistance.

3.2. LR interactions within the hypothalamic–gonadotropic axis

3.2.1. Hypothalamus

Neuroendocrine mechanism(s) through which leptin influences GnRH neuronal activity have not been completely elucidated. The LR is expressed abundantly within the hypotha-

lamus [48–53]; however, leptin may ultimately influence GnRH secretion mainly through interneuronal signaling mechanisms, as double-labeling studies conducted with rodents [32] and higher primates [54] have failed to demonstrate expression of LR on GnRH neurons. Watanobe [55] reported that leptin can act within the hypothalamus of rats to stimulate the release of GnRH, with highest sensitivity to leptin within the median eminence-arcuate nucleus (ME-ARC) in fasted rats. Based upon increases in both receptor mRNA and protein levels [56,57], fasting may enhance LR concentration in this region. Similarly, expression of the full-length LR in the ventromedial hypothalamus (VMH) was found to be much greater in feed-restricted than in full-fed ewes [50]. Negative energy balance induced by fasting appears to have presynaptic actions that are conveyed by a reduction in excitatory GABAergic drive onto GnRH neurons. Treatment with exogenous leptin prevents this reduction, indicating that leptin can act presynaptically to restore afferent GABAergic drive to GnRH neurons in fasted animals [58]. In morphological studies, populations of NPY neurons in the ARC co-express LR [56,59], and NPY appears to mediate a large part of leptin's neuroendocrine effects on both the GnRH-LH system [60,61] and feeding behavior [61]. Other downstream factors implicated in leptin action within the ARC include orexin [62] and agouti-related peptide (AgRP) [63], the latter of which is an endogenous antagonist of the melanocortin 3 (MC3-R) and 4 (MC4-R) receptors. The inhibitory effects of leptin on food intake are mediated primarily through these receptors. In addition, MC4-R may play a crucial role in leptin's ability to potentiate the LH surge in female rats [64], and disruption in the genes encoding MC3-R and MC4-R increases fat mass [65] and causes obesity in the mouse [66]. Although numerous other leptin-responsive, hypothalamic peptides have been identified, most do not appear to be related directly to GnRH secretion [e.g., α-MSH and cocaine-and amphetamine-regulated transcript (CART)]. A more recently identified peptide within the hypothalamus, galanin-like peptide (GALP), responds to leptin, stimulates the secretion of LH when administered centrally [67], and has been reported to either increase [68] or decrease food intake [69].

3.2.2. Adenohypophysis

In the ovine adenohypophysis, while LR is expressed in almost 90% of the gonadotropes in the pars tuberalis, it is observed in only about 30% of the gonadotropes in the pars distalis [70]. Moreover, approximately 25% of rat anterior pituitary cells, predominantly folliculostellate cells and corticotropes, express leptin [71]. This suggests a potential regulatory function of leptin in growth and differentiation of pituitary cells [12]. Moreover, direct effects of leptin at the adenohypophyseal level have been demonstrated in rodents [31], pigs [72], and cattle [35]. Intracellular mechanisms involved in the ability of leptin to regulate LH secretion at the adenohypophyseal level have not been thoroughly explored. However, there are several potential pathways through which these effects could occur, including effects on Ca^{2+} ion channels, an increase in the releasable pool of LH, and/or GnRH-receptor desensitization [73]. In porcine chromaffin cells, leptin caused a sustained increase of intracellular Ca^{2+} and activated inositol 1,4,5-triphosphate production [74], intracellular factors known to be associated with GnRH-receptor signaling and release of LH [75].

Pathways involved in the heightened sensitivity of the adenohypophysis of nutritionally stressed animals to leptin [35] have not been determined. However, the hypersecretion of LH observed in cattle in response to leptin as a consequence of short-term fasting is not

accompanied by a detectable increase in expression of LR mRNA in the anterior pituitary, nor a reduction in SOCS-3 mRNA expression [76]. To the contrary, SOCS-3 is generally elevated in adenohypophyseal tissue of fasted cows [76].

4. Leptin and the onset of puberty

4.1. Does leptin regulate the timing of puberty?

The onset of puberty is characterized by an acceleration of GnRH pulse generator activity, thereby increasing the pulsatile release of LH. Several studies have demonstrated an advancement of pubertal onset in female mice treated with leptin, and by indirect inference, an increase in GnRH and LH secretion. Treatments with leptin beginning on postnatal day 21 advanced vaginal opening by 1–4 d, increased weights of reproductive organs (ovaries, uterus, oviducts), and decreased latency to first mating [77]. In a similar study [78], mice given the same dose of leptin showed early onset of vaginal opening, vaginal estrus and vaginal cycling compared to vehicle-treated mice, without undergoing significant weight loss. Leptin has also been found to induce puberty in nutritionally growth-retarded female rats [79]. In contrast, Cheung et al. [80] found that leptin did not advance sexual maturation in the normally fed rat, but partially prevented the negative effects of food restriction on the timing of sexual maturation. Grauz et al. [81] found that leptin administration advanced sexual maturation in only 44% of female rats when starting leptin treatment at 29 d of age. This is in contrast to the findings of Almog et al. [82] in which administration of leptin to rats starting at 21 d of age advanced puberty in 100% of the animals. A “reassessment” of the role of leptin in sexual maturation in rats was later performed and reported by Cheung et al. [83]. Conclusions were that leptin is one of several permissive factors, whose presence is necessary, but alone is not sufficient, to initiate sexual maturation in rodents.

4.2. Leptin and puberty in cattle and sheep

In initial studies with heifers near the time of pubertal transition, we observed that fasting for 2 d markedly decreased leptin mRNA in adipose tissue, as well as circulating concentrations of leptin, and reduced the number of pulses of LH compared to non-fasted animals [36]. We then examined the pattern of adipose mRNA expression for leptin and serum concentrations of leptin during pubertal development [15], and correlated these measures with body weight and adiposity, proportions of bound/free leptin, and IGF-1 concentrations in serum [15,84]. Body weight accounted for the greatest variation associated with time of onset of puberty and was highly correlated with circulating leptin. Serum concentrations of leptin, IGF-1, and leptin gene expression increased as puberty approached in heifers reaching sexual maturation from early spring to mid-summer [15]. The increase in serum leptin was linear regardless of season of pubertal onset (Fig. 2). However, we found no evidence for the presence of leptin-binding proteins in bovine serum [84]. To the contrary, in humans, marked decreases in leptin-binding activity in serum accompany pubertal increases in circulating leptin, theoretically making it available to the full-length receptors [85]. Although earlier reports had indicated that leptin was capable of triggering pubertal

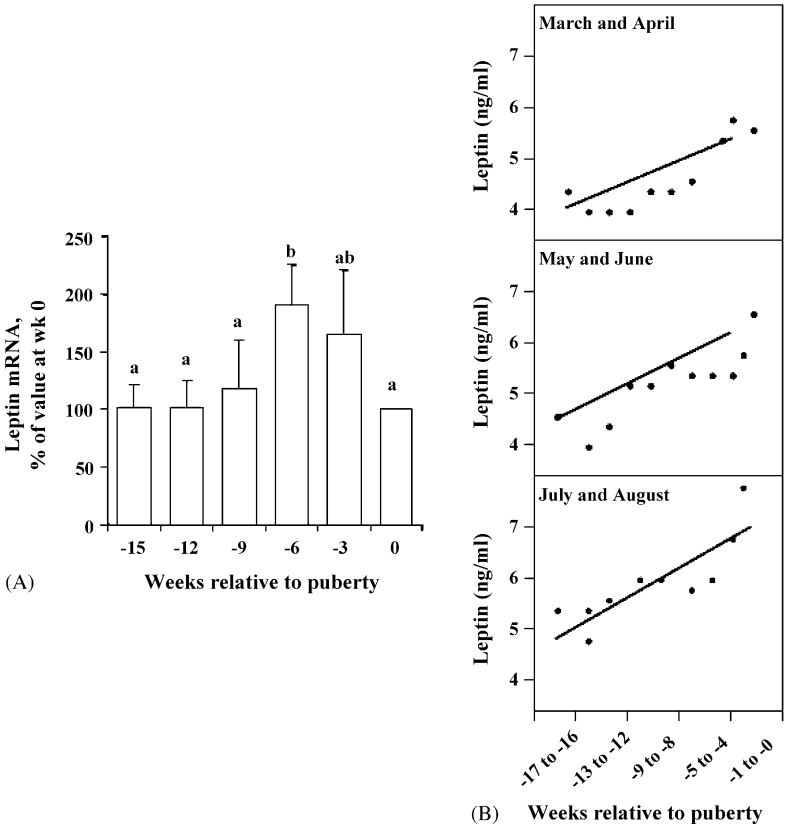

Fig. 2. Change in leptin gene expression during pubertal development (panel A) and serum leptin concentrations (panel B) in heifers that reached puberty from late winter/early spring to late summer. (Adapted with permission from [15].)

transition in rodents [77,78], we have found no evidence to support this hypothesis in our experiments with heifers. In those studies, neither chronic s.c. treatment of normally fed heifers [86], nor acute i.v. treatment of normal-growth or restricted growth heifers [45], were capable of accelerating the development of a sexually mature pattern of gonadotropin secretion. These observations are supported by studies in male lambs [87], demonstrating that leptin cannot drive GnRH secretion in individuals incapable of producing pulses due to developmental constraints. Additional studies sought to manipulate serum concentrations of leptin, and thus timing of puberty, during growth and development [88]. In one approach, we attempted to reduce the degree of adipose tissue accretion during pubertal development by suppressing the conversion of pre-adipocytes to adipocytes. Our objectives were to increase tissue contents of conjugated linoleic acid (CLA) through feeding a high linoleic acid diet. Theoretically, both rumen and milk contents of CLA can be increased several fold using this approach because CLA are produced as intermediate products of linoleic acid biohydrogenation in the rumen [89]. Although we were able to increase tissue content of

CLA somewhat by feeding such a diet to heifers between 4 months of age and puberty, we did not reduce adiposity or circulating concentrations of leptin and did not modify age at puberty [88].

5. Gonadal and embryonic effects of leptin

Not only does leptin participate in the control of gonadotropin secretion via its hypothalamic/pituitary actions, but circulating or locally produced leptin may also provide direct modulation of ovarian and testicular function. Bovine ovarian granulosa [90] and theca [91] cells have high affinity receptors for leptin. Expression of LR mRNA has been identified in adult human granulosa, theca and interstitial cells [92–94], in multiple tissues in the rat [48], and in the porcine ovary [49]. Leptin protein has been found in follicular fluid, with concentrations corresponding to those reported in serum [93], and leptin concentrations in peripheral blood vary throughout the menstrual cycle [95,96]. Leptin has been shown to inhibit IGF-1-mediated enhancement of FSH-stimulated estradiol synthesis by rat [97], human [98] and ovine granulosa cells [Zieba et al., unpublished data], and LH-stimulated androgen synthesis by bovine theca cells [91]. Recent studies have shown that both in vivo administration of leptin to immature gonadotropin-primed rats, and in vitro exposure of perifused rat ovaries to elevated concentrations of leptin, can lead to a marked decline in the number of ovulated oocytes [99]. However, relatively little research has focused on receptor-mediated events in maturing oocytes and pre-implantation embryos. LR mRNA and protein are expressed in mouse oocytes [100–102], and leptin induces tyrosine phosphorylation of STAT3, a major intracellular leptin signal transduction protein in mouse metaphase II stage oocytes [101]. Moreover, in embryo culture media, leptin promoted the development of embryos from the two-cell stage to blastocysts, fully expanded blastocysts, and hatched blastocysts [100]. However, exposure of porcine or ovine oocytes to leptin during in vitro maturation and subsequent embryo culture after IVF resulted in the formation of fewer blastocysts relative to controls ([103]; Zieba et al., unpublished data). Both the long (OB-Rb) and short isoforms (OB-Ra) of the LR are expressed in rodent Leydig cells [104], indicating potential effects of leptin on testis function. Moreover, leptin inhibits human chorionic gonadotropin-stimulated testosterone secretion from rat testicular explants [105].

6. Metabolic adaptation and endocrine responses to leptin

The role of leptin as a mediator of physiological responses to nutritional stress has received significant attention in this review, particularly in relation to reproduction. Food deprivation results in a fall in circulating leptin, and if unmitigated, results in a cessation of reproduction, a survival mechanism that is achieved relatively quickly in monogastrics and less quickly in ruminants [106,107]. While serving as a communications link between nutritional status and reproduction, leptin ultimately supports metabolic homeorhesis by influencing eating behavior [108]. Moreover, centrally mediated signals within the ARC drive changes within other endocrine systems, including the somatotrophic, pancreatic, thyrotrophic, and adrenal axes. These interrelationships have been summarized extensively

for laboratory animals by others and will not be discussed in detail here [60,108,109]. However, recent investigations in cattle, sheep, and pigs have provided some insight into the role of leptin in regulating metabolic endocrinology in farm species.

6.1. Somatotrophic axis

In pigs, leptin is a potent anorexigenic agent, stimulates GH secretion in an inverse, dose-dependent manner in full-fed animals, and suppresses GHRH-mediated release of GH [110]. Interestingly, GHRH appears to down-regulate the expression of the long form of the LR in pigs, suggesting a reciprocal relationship between leptin and GHRH in regulating GH secretion [111]. Direct effects of leptin on GH release in that species was attributed to a potential decrease in NPY release. Paradoxically, NPY actually stimulates GH release in ruminants [112–114], and pretreatment of cows with leptin blocks GH release in response to a simulated, post-secretory rise of NPY administered into the IIIV [114]. The seemingly counterintuitive ability of both NPY and leptin to stimulate GH secretion in ruminants has not been fully explained. Nonetheless, similar to leptin's effects on secretion of LH, ruminants are more likely to respond to leptin with increased basal release of GH when under nutritional stress. For example, continuous ICV infusion of recombinant hleptin had no effect on circulating concentrations of GH in ovariectomized, full-fed sheep [41], but increased circulating GH in chronically undernourished sheep [43] and in fasted heifers [42]. The ability to detect such effects is related to some extent to the timing, duration, and dose of leptin [115]. In gonadectomized wethers, continuous treatment with leptin, as

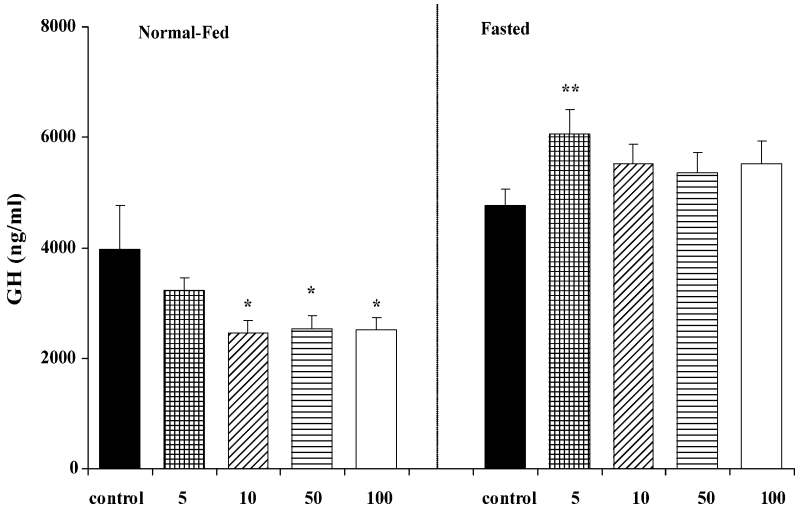

Fig. 3. Effects of oleptin (0, 5, 10, 50 and 100 ng/ml) on mean (±S.E.M.) concentrations of growth hormone (GH) in perifusion media of adenohypophyseal (AP) explants collected from normal-fed and fasted mature ovarectomized cows: (*) and (**) denote differences from controls ($P < 0.01$ and $P < 0.002$, respectively). (Adapted with permission from [115].)

opposed to chronic treatment beginning at the onset of fasting [43], did not stimulate the secretion of GH [37]. However, similar to results obtained with LH [35], basal secretion of GH from anterior pituitary explants of fasted, but not normal-fed cows, was potentiated acutely by low, but not high, doses of leptin [115]. In fact, doses of oleptin above 5 ng/ml suppressed basal release of GH in anterior pituitary explants from full-fed cows (Fig. 3). However, there is evidence that leptin positively modulates anterior pituitary synthesis and secretion of LH and GH in full-fed ruminants [34,115]. Although leptin-mediated increases in basal (spontaneous) secretion of LH and GH were observed only in explants from fasted cows, both GnRH-mediated release of LH [34] and GHRH-mediated release of GH [115] were enhanced in anterior pituitary explants from full-fed cattle, and only explants from full-fed cows were able to respond to GHRH with an increase in GH release (Fig. 4).

6.2. *Hepatic and pancreatic responses to leptin*

Circulating concentrations of IGF-I and insulin consistently decrease in sheep and cattle during fasting [36,116]. Although leptin appears to have no effect on circulating

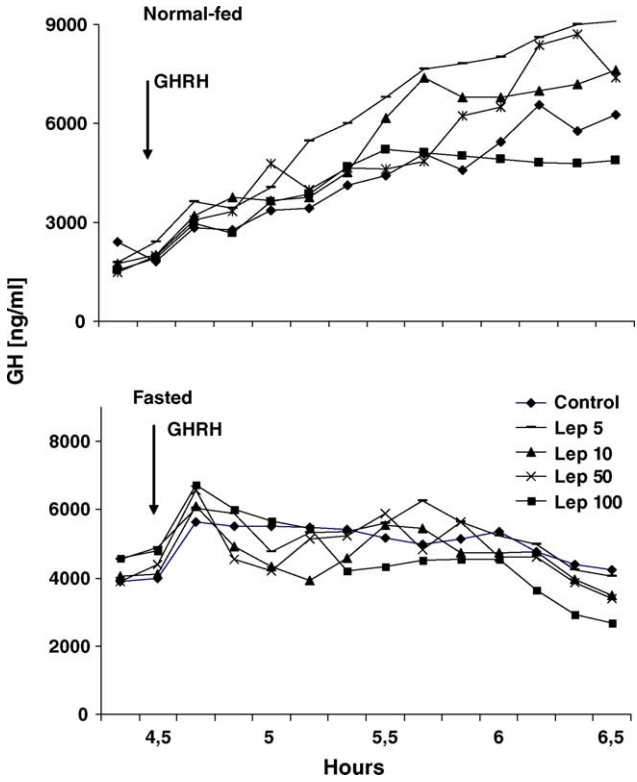

Fig. 4. Temporal patterns of growth hormone (GH) release after growth hormone releasing hormone (GHRH) stimulation in perifusion media of adenohypophyseal (AP) collected from explants from normal-fed and fasted mature, ovariectomized cows.

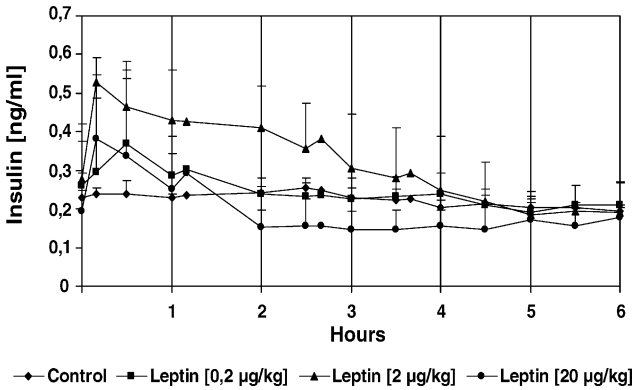

Fig. 5. Mean (±S.E.M.) concentrations of insulin in ovariectomized cows on day 2 of fasting before and after single i.v. injections of saline or recombinant oleptin. Plasma insulin concentrations increased ($P < 0.01$) in all leptin-treated groups and reached their highest concentration during the first hour. The stimulation of insulin secretion was greatest ($P < 0.001$) at the intermediate dose of 2.0 μg/kg relative to the control group and other leptin-treated groups. This effect persisted for 3 h. (Adapted with permission from [34].)

IGF-1 in cattle [33,35,42], both ICV [33] and peripheral infusions [34] of leptin stimulate increases in circulating insulin, and these effects are dependent upon the dose of leptin employed [34] (Fig. 5). A single, low-dose (2.0 μg/kg) injection of leptin into fasted cows elevated serum insulin for over 3 h, whereas lower (0.2 μg/kg) and higher (20 μg/kg) doses had only brief effects. The presence of LRs in pancreatic islets [117] indicates that leptin can regulate directly the secretion of insulin. In rodents, leptin either failed to affect basal or glucose-induced insulin secretion [118,119], stimulated insulin release [120,121], or suppressed its release [117,122,123]. We interpret our results in cows to mean that an increase in circulating concentrations of leptin, physiologically a product of food intake and positive energy balance, acts as a signal to normalize pancreatic insulin secretion following nutritional restriction. Failure of the pancreas to respond to leptin in other experimental models, particularly animals under normal feeding conditions, may be related to a state of leptin resistance [33,34]. Tissues exposed to relatively large concentrations of leptin tend to accumulate excessive amounts of suppressors of cytokine signaling, which can mediate leptin resistance [21,124,125].

7. Physiological consequences of genotype

The pursuit of marker-assisted selection strategies to identify physiological traits of importance to medicine and agriculture has resulted in the identification of a number of genotypic polymorphisms related to the leptin gene. Although an in-depth discussion of this area is beyond the scope of this review, its importance, particularly in animal agriculture, for predicting or selecting for economically important genetic traits should not be underestimated. An early example of this strategy was the identification of the restric-

tion fragment length polymorphism (RFLP), Sau3AI within the bovine leptin gene [126]. Three genotypes from two alleles were reported in eight breeds of cattle, and it was proposed that these alleles (A and B) could represent differences in adiposity and physiological characters related to adiposity. In one survey of cattle in Northern Mexico and the southwestern U.S., it was determined that the relatively high frequency of A allele, and conversely, low frequency of the B allele, probably rendered the SAU3AI-RFLP uninformative for use in selection strategies [127]. This RFLP and two others were examined by Liefers et al. [128] in dairy cattle. The SAU3AI RFLP (termed RFPL1 in that study) was found to be related to milk yield; however, again the frequency of the B allele probably precludes it from being useful for selection. More recently, a single nucleotide polymorphism (SNP) [129] has been identified in which presence of the dominant t allele (ct or tt) was related to increased fat deposition [130], increased dry matter intake, and greater milk and milk protein yield [129]. Cattle bearing the t allele may exhibit a form of resistance in which the LR does not recognize the modified form of leptin resulting from this mutation. Currently, this SNP, and the physiology surrounding it, are the focus of a major proprietary development effort in North America (Igenity^{TM}, Merial Corp, Duluth, GA) in relation to the identification of genotypic variance in carcass quality in beef cattle and milk yield in dairy cattle. The frequency of the alleles in various beef cattle breeds and DNA analyses to determine genotypes related to these alleles has moved leptin physiology into the realm of functional genomics. Earlier studies in beef cattle had already provided physiological evidence that relatively higher levels of circulating leptin, a probable indicator of leptin resistance, are related to modifications in carcass adiposity [131]. However, it has not been reported whether cattle with circulating concentrations of leptin much greater than the average of the population represent genetic variances in genes for leptin or the LR.

8. Summary and conclusions

During the last 10 years, the study of leptin and it roles in reproduction and metabolism of animals has taken on nearly gigantic proportions. Its contributions as a hormone acting at multiple loci have now been clearly established, including effects at the hypothalamus, adenohypophysis, pancreas, and gonads. These actions involve pathways that regulate appetite and energy expenditure, as well as the secretion of reproductive and metabolic hormones. All of these regulatory pathways involve activation of the LR, but also include many downstream molecules that modulate both sensitivity and resistance to leptin. In addition, collective evidence across several species supports the idea that leptin plays a passive or permissive, rather than causal, role in timing the process of pubertal maturation. Finally, one of the most intriguing and valuable discoveries has been that the hypothalamic–adenohypophyseal axis becomes hypersensitive to leptin during fasting, with an ability of leptin to stimulate LH and GH secretion by direct effects at both the hypothalamic and adenohypophyseal levels. Much additional work will be necessary to delineate fully the role of leptin in the variety of physiological systems with which it is involved, and to develop managerial, pharmacological, and genetic strategies for exploiting those roles in medicine and agriculture.

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