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Original Research Article

Leptin inhibits the reproductive axis in adult male Syrian hamsters exposed to long and short photoperiod

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ABSTRACT

The aim of the study was to investigate the effects of acute leptin treatment of adult Syrian hamsters exposed to a long (LP, eugonadal males) and short photoperiod (SP, hypogonadal males). Animals were exposed to LP (L:D 14:10) or SP (L:D 10:14) for 10 weeks. Afterwards, both LP and SP hamsters were allocated to a control (SP-C, LP-C) or leptin-treated group (SP 3, SP 10, SP 30 or LP3, LP 10, LP 30). One hour before sacrifice, a single dose of leptin (3, 10 or 30 μg/kg) or vehicle was administered (i.p.) to the males. Testis weight, serum and pituitary luteinizing hormone (LH) concentrations, as well as the hypothalamic concentration of gonadotropin-releasing hormone (GnRH) were recorded. Histological analysis of the testis was performed and GnRH concentration in the culture medium of hypothalamic explants was examined. A dramatic regression of testicular weight and histological atrophy of seminiferous tubules, as well as a decrease in serum and pituitary LH concentrations were found in SP males. All doses of leptin significantly reduced serum LH levels and medium GnRH concentrations in both photoperiod groups. Pituitary LH and hypothalamic GnRH concentrations were not affected by leptin. In conclusion, we demonstrated that leptin inhibited the reproductive axis of Syrian male hamsters exposed to LP and SP and fed *ad libitum*.

1. Introduction

Syrian hamsters (*Mesocricetus auratus*) are seasonal breeders and their exposure to a short photoperiod (SP; less than 12.5 h light/day) causes a marked reduction in testicular mass and the diameter of seminiferous tubules [1,2]. In the exposed animals, spermatogenesis and sperm count decline sharply, ceasing entirely after 10 weeks of the SP exposure [3]. This is accompanied by a decrease in the activity of the

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hypothalamus–pituitary axis and in hypothalamic norepinephrine turnover. Thus, the reproductive changes associated with photoperiod-induced gonadal regression seem to be secondary to the changes in hypothalamic function [4]. The regression appears to be mediated by the hypothalamic dorsomedial (DMH) and ventromedial (VMH) hypothalamic nuclei [5,6]. Lesions of these areas may block the gonadal regression induced by SP in hamsters [7] and inhibit the SP-induced weight gain. It appears, however, that it is the alteration in a pulsatile GnRH secretion rather than synthesis which is the most relevant aspect of the hamster exposure to short photoperiod [8–10]. A short photoperiod has been previously found to decrease the number of GnRH pituitary receptors [11].

Leptin is a hormone secreted by adipocytes that controls metabolic status, depot fat and appetite [12]. It functions as a neuroendocrine signal in the hypothalamus [13–15] and is involved in the regulation of gonadal processes. Leptin activates VMH and DMH neurons [16], maintains sexual maturation of female rats [17] and advances the onset of puberty in mice [14]. We have previously shown that leptin stimulated the reproductive axis during prepubertal and peripubertal stages in female rats [18,19]. Moreover, leptin was reported to preserve cyclicity and reproductive functions in humans and rodents [20–22], as well as to prevent hypogonadism induced by fasting in primates [23] and reinitiate the suppressed reproductive function in ob/ob mice [24].

We demonstrated that a single acute injection of leptin inhibits rather than stimulates the hypothalamo–pituitary–gonadal axis in normal adult male rats [25]. Although these results were derived from experiments performed on non-seasonal breeders, leptin is known to affect the reproductive axis also in seasonal breeders *e.g.*, sheep [26–28]. In hamsters, the expression of the leptin gene in adipose tissues was affected by the photoperiod, and seasonal changes in fatness and metabolism occurred synchronously with the activation or supression of reproduction [29,30]. Daily fluctuations in leptin serum levels are also affected by the photoperiod [31]. Exposure to a long photoperiod enhanced *leptin* expression in white adipose tissue [29] and increased leptin serum concentration [32]. Similar seasonal changes in serum leptin and adiposity have also been reported in sheep [33]. In mammals, the photoperiod is communicated with the central nervous system (CNS) *via* melatonin, secretion of which is regulated by the length of night [34], reflecting both daily and annual changes in the photoperiod. This explains the role of melatonin in the regulation of mammalian circadian and seasonal rhythms [35,36]. Seasonal changes in reproductive responses (*i.e.*, gonadal regression) are governed by the interaction between melatonin and the hypothalamic–pituitary–gonadal axis [37]. The goal of the study was to examine the effects of acute leptin treatment on the neuroendocrine reproductive function of adult male Syrian hamsters (seasonal breeders). The parameters examined in hamsters treated with three doses of leptin and exposed to a long photoperiod (LP; eugonadal males) or SP (hypogonadal males) included serum LH concentration as well as pituitary LH and hypothalamic GnRH contents. In addition, medium GnRH concentration was examined after *in vitro* culture of hamster hypothami.

2. Materials and methods

2.1. Animals

Adult male Syrian hamsters (*M. auratus*; 3 month-old) were kept under controlled temperature and artificial light conditions (24 ± 2 °C, 14:10 light-dark cycles with lights off at 20:00) and were housed in groups. All hamsters had free access to food and water until sacrifice. Adequate measures were taken to minimize pain and discomfort of animals. All experiments were performed in accordance with the principles and procedures outlined in European Communities Council Directives (86/609/EEC) and FRAME guidelines (*FRAME Reduction Committee November 1999*).

2.2. Experimental protocol

Before the experiment the animals were divided into two groups. One group was exposed to LP (14/10 h; lights from 6.00 to 20.00) and the other to SP (10:14 h; lights from 8.00 to 18:00) for 10 weeks (period required for maximal gonadal regression in the SP group). Then, hamsters from each photoperiod group were designated to a control group, receiving single saline injection (SP-C or LP-C) or to one of the three experimental groups receiving 3, 10 or 30 μg/kg of leptin (Sigma–Aldrich, St. Louis, MO, USA) in a single i.p. injection (SP-3, LP-3, SP-10, LP-10, SP-30, LP-30). Each group consisted of 12–14 animals. The treatments were administered during the third hour of the light phase of the last day of the 10-week treatment period. All hamsters were sacrificed by decapitation 60 min after the treatment injection. Trunk blood was collected and centrifuged, and serum samples were kept at −70 °C until LH determination.

2.3. Tissue processing and hypothalamic incubation

The testes and seminal vesicles were dissected and weighed. Only the LP-C and SP-C testes were processed for histological examination. Pituitary glands and anteromediobasal hypothalami (AMBH) were immediately dissected with the help of a stereomicroscope. The collected hypothalamic tissues were bordered laterally by the hypothalamic sulci, rostrally by a line about 3 mm from the optic chiasm, and caudally by the mammillary bodies; the collected tissues had a thickness of 3–4 mm. Neurohypophysis was separated from adenohypophysis. All collected tissues were weighed and processed in respective buffers. Anterior pituitaries were homogenized in 1 ml isotonic phosphate buffer (disodium phosphate 0.005 M, monosodium phosphate 0.005 M, sodium chloride 0.15 M), pH: 7.5. The supernatant was diluted 1:150 in buffer and kept at −70 °C until LH determination. In the first experiment (GnRH determination), the hypothalamic tissues (0.01 mg) were homogenized in 20 μl of 0.1 N hydrochloric acid/mg of tissue using an ice-cold glass Potter homogenizer. The homogenate was centrifuged (11 300 g; 2 min), the supernatant was diluted 1:1 with phosphate buffer and frozen at −70 °C until the GnRH assay. In the second experiment (*in vitro* GnRH release), the AMBH were used immediately after sacrifice [25,38]. After dissection, the AMBH were placed in plastic chambers

containing 300 μl of Earle's medium with glucose (1 mg/ml) and bacitracin (20 mM), pH 7.4. Hypothalamic samples of similar weight were used in each incubation. Each chamber was kept in a Dubnoff shaker (37 °C; 95% O_2, 5% CO_2) with constant shaking (60 cycles/min). After pre-incubation (20 min), the medium was changed and the tissues were incubated for subsequent 60 min. Then, the medium was collected and frozen at −70 °C for GnRH determination.

2.4. Hormone concentration determination

Serum (ng/ml) and pituitary (ng/mg) luteinizing hormone (LH) concentrations were determined in duplicate by radioimmunoassay (RIA) with a purchased kit (NIAMDD, Bethesda, MD, USA). Intra and inter-assay coefficients of variation were 7% and 10%, respectively; detection limit was 0.2 ng/ml and LH antibodies (final concentration 1:70 000) were from the National Institute of Diabetes and Digestive and renal Disease (NIDDK-anti-rLH-S-11). GnRH concentration was determined in duplicate by RIA using a specific antibody (with a final concentration 1:30 000) (Dr. W. Wuttke, Goettingen, Germany). I^{125}GnRH was purchased from New England Nuclear (Boston, MA, USA) and synthetic GnRH (Peninsula Laboratories, Belmont, CA, USA) served as standard. For the precipitation of the immune complex, cold ethyl alcohol was used. The detection limit was 0.2 pg/100 μl. Intra- and inter-assay coefficients of variation were 7% and 12%, respectively. GnRH data were expressed as pg/AMBH.

2.5. Statistical analysis

The results are expressed as means ± SEM. Significance was assessed by two-way analysis of variance (ANOVA) followed by the Dunnet test. For comparison of weight data a unpaired Student *t*-test was used (SPSS v10).

3. Results

After 10 weeks of exposure to the two photoperiods, the mean body weight (BW) did not differ between the SP and LP groups. However, the weight of the testis and seminal vesicles was dramatically lower ($p < 0.001$) in SP than in LP hamsters (Table 1). The decrease ($p < 0.001$) in serum LH concentration (Fig. 1, open bars) and pituitary LH content (Table 2) in the SP group was also significant. These changes were consistent with the regression of seminiferous tubules' epithelium observed in the SP-C group when compared to the LP-C group (Fig. 2).

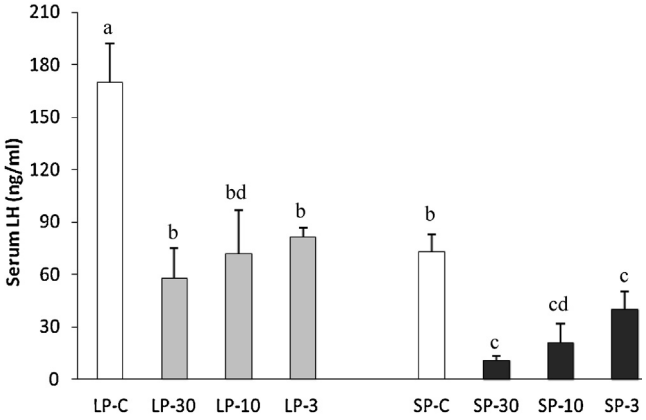

Fig. 1 – Serum LH concentration (mean ± SEM) in hamsters exposed to long (LP, gray bars) or short photoperiod (SP, black bars) and treated with leptin. In each photoperiod group three doses of leptin were applied: 3 (LP-3 and SP-3), 10 (LP-10 and SP-10) or 30 (LP-30 and SP-30) μg/kg. For more details see the text. Different superscripts depict significant differences ($p < 0.05$); SP-C and LP-C (white bars): control groups without leptin.

In both photoperiod groups, all doses of leptin significantly reduced serum LH concentration (LP-30 and SP-30: $p < 0.001$, LP-10 and SP-10: $p < 0.01$, LP-3 and SP-3: $p < 0.05$). In addition, leptin administration produced lower ($p < 0.05$) serum LH levels in SP-30 and SP-3 hamsters than in their LP counterparts. Serum LH did not differ among the leptin sub-groups within each photoperiod group (Fig. 1). Pituitary LH and hypothalamic GnRH concentrations were not affected by leptin administration in any of the photoperiod groups (Table 2).

In another experiment, we determined GnRH concentration in medium collected after the culture of hypothalamic explants (AMBH), which were harvested from hamsters exposed to LP or SP (Fig. 3). In both photoperiod groups, all leptin doses significantly decreased medium GnRH concentration (LP-30 and SP-30: $p < 0.001$, LP-10 and SP-10: $p < 0.01$, LP-3 and SP-3: $p < 0.05$).

4. Discussion

In the present study, 10 week-exposure to SP induced gonadal regression in adult Syrian hamster males. The regression of

Table 1 – Weight of body, testes and seminal vesicles in hamsters exposed to long photoperiod (LP) or short photoperiod (SP).

	Unit	LP	SP
Initial BW	g	158.4 ± 5.1	166.7 ± 3.2
Final BW	g	186.4 ± 5.5	195.3 ± 6.7
Final testis weight	mg/100 g BW	2157.6 ± 62.1	295.9 ± 66.1*
Final seminal vesicles weight	mg/100 g BW	665.4 ± 93.6	230.8 ± 37.0*

* $p < 0.001$; BW: body weight.

Table 2 – Effects of acute leptin administration on pituitary LH concentration (ng/mg tissue) and hypothalamic GnRH concentration (pg/mg tissue) in male hamsters exposed to long photoperiod (LP) or short photoperiod (SP).

	Pituitary LH concentration	Hypothalamic GnRH concentration
LP-C	1286.7 ± 43.6	0.97 ± 0.12
LP-30	1558.5 ± 409.5	1.32 ± 0.06
LP-10	1386.3 ± 90.8	0.68 ± 0.12
LP-3	876.1 ± 110.5	0.58 ± 0.06
SP-C	436.1 ± 41.4*	1.12 ± 0.11
SP-30	697.7 ± 143.3*	1.54 ± 0.17
SP-10	395.5 ± 24.2*	1.11 ± 0.06
SP-3	577.1 ± 76.7*	0.65 ± 0.12

LP-C and SP-C: control groups without leptin; LP-30 and SP-30. 30 μg/kg; LP-10 and SP-10: 10 μg/kg; LP-3 and SP-3: 3 μg/kg.
* $p < 0.05$: different from the respective LP group.

the seminiferous tubules' epithelium and the decrease in serum and pituitary LH concentrations in the males were consistent with the SP-induced suppression of reproductive functions in male hamsters. Leptin administration did not abolish the inhibitory effect of SP on the examined reproductive parameters. The acute administration of leptin produced the inhibition of the reproductive axis in LP and SP animals. In previous studies leptin also did not reverse the reproductive suppression induced by SP exposure in Siberian hamsters [39]. Moreover, we demonstrated that the acute administration of leptin to adult male hamsters exposed to LP or SP decreased *in vitro* GnRH secretion by hypothalamic explants. These results clearly demonstrated that leptin inhibited the hypothalamus–pituitary–gonadal axis in animals exposed to the short and long photoperiod. However, neither pituitary LH nor hypothalamic GnRH concentration was affected by the leptin treatment. It is possible that the time between leptin administration and sacrifice of the animals was too short to observe changes in hormone synthesis.

Leptin plays a key role in maintaining body weight homeostasis and is a relevant neuroendocrine mediator in the regulation of reproductive functions [12,17,21,40–42]. The inhibitory effects of leptin on the reproductive axis results from its effect on the hypothalamus and is evidenced by a decrease in GnRH secretion. Such inhibition might result from an inhibitory action of leptin on food intake, although the time between leptin administration and tissue sampling was too short (one hour) for the changes to occur. Previously, we demonstrated that leptin (30, 100 and 300 μg/kg) stimulated gonadal function in pre- and peripubertal females and males of Wistar rats [18,43], but inhibited the reproductive axis in adult males [25]. An inhibitory effect of leptin has also been reported on *in vitro* LH and FSH hemi-pituitary secretion in male adult rats [33,44].

In the current study, peripheral leptin administration inhibited the reproductive axis in adult, *ad libitum* fed hamster males. Similarly, the intracerebroventricular (icv) infusion of leptin reduced the LH pulsatile frequency and decreased voluntary food intake in *ad libitum* fed adult rams (also seasonal breeders) [45]. It is well known that malnutrition, acting at a neuroendocrine level, produces an inhibition of the reproductive axis. In under-fed animals, both icv and peripheral leptin administration was reported to reverse the inhibition observed in the gonadal axis [26,28]. The stimulatory effect of leptin was not demonstrated in well-fed sheep [27]. Thus, the paradoxical inhibitory effect of leptin on the reproductive axis appears to depend on the metabolic status of the animal.

In conclusion, in the current paper, we demonstrated that leptin had an inhibitory effect on the reproductive axis of adult male Syrian hamsters that were fed *ad libitum* and were maintained under both long (eugonadal males) and short

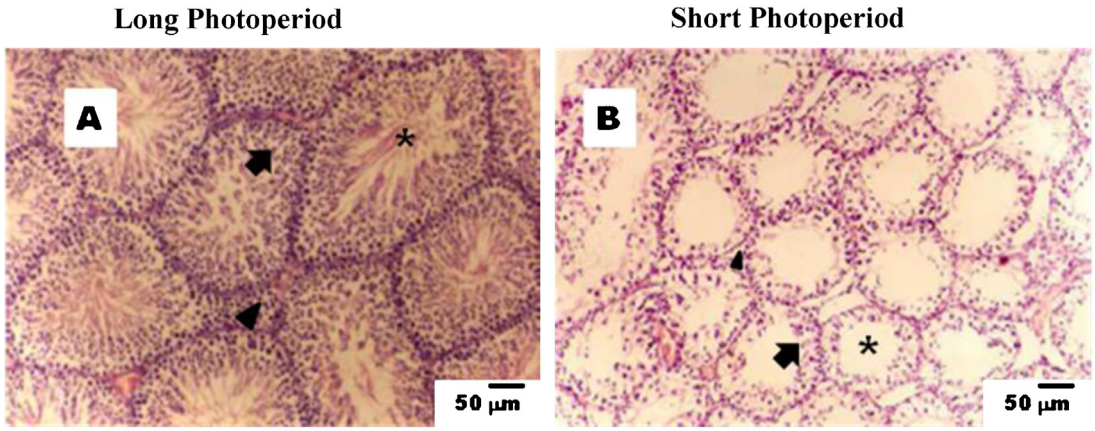

Fig. 2 – Histological images of the seminiferous tubule epithelium of testes harvested from hamsters exposed to long (A) or short (B) photoperiod. Asterisks indicate the lumen of the seminiferous tubule, arrows point to spermatogenic cells and arrowheads point to interstitial tissue (hematoxylin–eosin; 40× magnification).

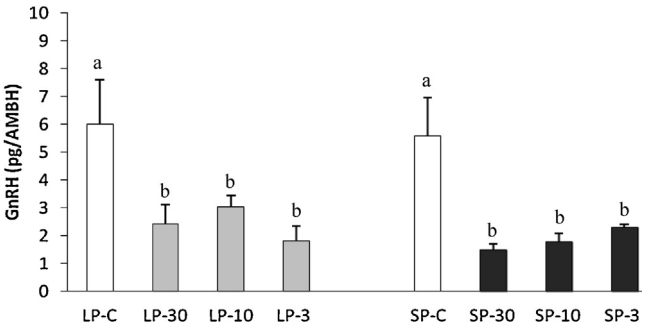

Fig. 3 – GnRH concentration (mean ± SEM) in medium collected after culture of hypothalamic explants harvested from hamsters exposed to long (LP, gray bars) or short photoperiod (SP, black bars). The hamsters in each photoperiod group received three doses of leptin: 3 (LP-3 and SP-3), 10 (LP-10 and SP-10) or 30 (LP-30 and SP-30) μg/kg. For more details see the text. Different superscripts depict significant differences ($p < 0.05$); SP-C and LP-C (white bars): control groups without leptin.

photoperiod (hypogonadal males). This inhibition was mediated by hypothalamic neuroendocrine mechanisms that regulate gonadal functions.

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REFERENCES

[1] Anderson KD, Turek FW. Photoperiodic control of testicular size in the golden hamster: lack of a role for the deep pineal gland. Journal Pineal Research 1985;2:99–107.
[2] Sisk CL, Turek FW. Reproductive responsiveness to short photoperiod develops postnatally in male golden hamsters. Journal of Andrology 1987;8:91–6.
[3] Breckon G, Cawood AH. Photoperiodic control of meiosis in the male Syrian hamster (*Mesocricetus auratus*). Journal of Reproduction and Fertility 1985;75:177–81.
[4] Steger RW, Bartke A, Goldman BD. Alterations in neuroendocrine function during photoperiod induced testicular atrophy and recrudescence in the golden hamster. Biology of Reproduction 1982;26:437–44.
[5] Bae HH, Mangels RA, Cho BS, Dark J, Yellon SM, Zucker I. Ventromedial hypothalamic mediation of photoperiodic gonadal responses in male Syrian hamsters. Journal of Biological Rhythms 1999;14:391–401.
[6] Maywood S, Bittman EL, Hastings MH. Lesions of the melatonin- and androgen-responsive tissue of the dorsomedial nucleus of the hypothalamus block the gonadal response of male Syrian hamsters to programmed infusions of melatonin. Biology of Reproduction 1996;54:470–7.
[7] Bartness TJ, Bittman EL, Wade GN. Paraventricular nucleus lesions exaggerate dietary obesity but block photoperiod-induced weight gains and suspension of estrous cyclist in Syrian hamsters. Brain Research Bulletin 1985;14:427–30.
[8] Brown DI, Garyfallou VT, Urbanski UB. Photoperiodic modulation of GnRH mRNA in the male Syrian hamster. Brain Research Molecular Brain Research 2001;89:119–25.
[9] Caillol M, Rossano B, Martinet L. Effect of short photoperiods on the in vitro GnRH release by hypothalamic explants in intact and castrated male Syrian hamsters: relation to testicular regression and recrudescence. Journal of Neuroendocrinology 1998;10:343–51.
[10] Swann JM, Turek FW. Transfer from long to short days reduces the frequency of pulsatile luteinizing hormone release in intact but not in castrated male golden hamsters. Neuroendocrinology 1988;47:343–9.
[11] Pieper DR. Effects of photoperiod, castration, and gonadotropin-releasing hormone (GnRH) on the number of GnRH receptors in male golden hamsters. Endocrinology 1984;115:1857–62.
[12] Friedman JM, Halaas JL. Leptin and the regulaton of body weight in mammals. Nature 1998;395:770–93.
[13] Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, et al. Leptin is a metabolic signal to the reproductive system. Endocrinology 1996;137:3144–7.
[14] Chehab FF, Mounzih K, Lu R, Lim ME. Early onset of reproductive function in normal female mice treated with leptin. Science 1997;275:88–90.
[15] Yura S, Ogawa Y, Sagawa N, Masuzaki H, Itoh H, Ebihara K, et al. Accelerated puberty and late-onset hypothalamic hypogonadism in female transgenic skinny mice overexpressing leptin. Journal of Clinical Investigation 2000;105:749–55.
[16] Elmquist JK, Ahima RS, Elias CF, Flier JF, Saper CB. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. Proceedings of the National Academy of Sciences of the United States of America 1998;95:741–6.
[17] Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology 1997;138:855–8.
[18] Ponzo OJ, Szwarcfarb B, Rondina D, Carbone S, Reynoso R, Scacchi P, et al. Changes in the sensitivity of gonadotrophin axis to leptin during sexual maturation in female rats. Neuroendocrinology Letters 2001;22:427–31.
[19] Reynoso R, Ponzo O, Szwarcfarb B, Rondina D, Carbone S, Rimoldi G, et al. Effect of leptin on hypothalamic release of GnRH and neurotransmitter amino acid during sexual maturation in female rats. Experimental and Clinical Endocrinology and Diabetes 2003;111:274–7.
[20] Blum WF, Englaro P, Hanistsh S, Juul A, Hertel NT, Muller J, et al. Plasma leptin levels in children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage and testosterone. Journal of Clinical Endocrinology and Metabolism 1997;82:2904–10.
[21] Carro E, Pinilla L, Seoane L, Considine R, Aguilar E, Casanueva F, et al. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology 1997;66:375–7.
[22] Laughlin GA, Yen SSC. Hypoleptinemia in women and athletes: absence of a diurnal rhythm with amenorrhea. Journal of Clinical Endocrinology and Metabolism 1997;82:318–21.
[23] Finn PD, Cunningham MJ, Pau KY, Spies HG, Clifton DK, Steiner RA. The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology 1998;139:4652–62.

[24] Mounzih K, Lu R, Chehab FF. Leptin treatment rescues the sterility of genetically obses ob/ob males. Endocrinology 1997;138:1190–3.
[25] Ponzo OJ, Reynoso R, Rondina D, Szwarcfarb B, Carbone S, Scacchi P, et al. Effect of leptin on LH levels and hypothalamic release of GnRH. Its relationship with the hypothalamic neurotransmitter amino acids system in adult male rats. Endocrine Research 2004;30:179–87.
[26] Adam CL, Archer ZA, Miller DW. Leptin actions on the reproductive neuroendocrine axis in sheep. Reproduction 2003;61(Suppl.):283–97.
[27] Henry BA, Goding JW, Tilbrook AJ, Dunshea FR, Clarke IJ. Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. Journal of Endocrinology 2001;168:67–77.
[28] Polkowska J, Wójcik-Gładysz A, Wańkowska M. The effect of intracerebroventricular infusions of leptin on the immunoreactivity of neuropeptide Y and gonadotrophin releasing hormone neurons in the hypothalamus of prepubertal sheep in conditions of short fasting. Journal of Chemical Neuroanatomy 2006;32:65–73.
[29] Klingenspor M, Dickopp A, Heldmaier G, Klaus S. Short photoperiod reduces leptin gene expression in brown and white adipose tissue of Djungarian hamsters. FEBS Letters 1996;399:290–4.
[30] Klingenspor M, Niggermann H, Heldmaier G. Modulation of leptin sensitivity by short photoperiod acclimation in the Djungarian hamster, *Phodopus sungorus*. Journal of Comparative Physiology 2000;170:37–43.
[31] Gunduz B. Daily rhythm in serum melatonin and leptin levels in the Syrian hamster (*Mesocricetus auratus*). Comparative Biochemistry and Physiology Part A 2002;32:393–401.
[32] Horton TH, Buxton OM, Losee-Olson S, Turek FW. Twenty-four-hour profiles of serum leptin in siberian and golden hamsters: photoperiodic and diurnal variations. Hormone and Behaviour 2000;37:388–98.
[33] Marie M, Findaly PA, Thomas L, Adam CL. Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. Journal of Endocrinology 2001;170:277–86.
[34] Goldman BD. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. Journal of Biological Rhythms 2001;16:283–301.
[35] Cassone VM. Effects of melatonin on vertebrate circadian systems. Trends in Neurosciences 1990;13:457–64.
[36] Reiter RJ. The melatonin rhythm: both a clock and a calendar. Experientia 1993;49:654–64.
[37] Mason AO, Duffy S, Zhao S, Ubuka T, Bentley GE, Tsutsui K, et al. Photoperiod and reproductive condition are associated with changes in RF amide-related peptide (RFRP) expression in Syrian hamsters (*Mesocricetus auratus*). Journal of Biological Rhythms 2010;25:176–85.
[38] Ponzo OJ, Rondina D, Szwarcfarb B, Carbone S, Moguilevsky JA, Scacchi P. Effects of aproteic diet on hypothalamic–pituitary–gonadal regulation in the male rat. Neuroendocrinology Letters 1999;20:19–24.
[39] Atcha Z, Cagampang FRA, Stirland JA, Morris ID, Brooks AN, Ebling FJP, et al. Leptin acts on metabolism in a photoperiod dependent manner, but has no effect on reproductive function in the seasonally breeding Siberian hamster (phodopus sungorus). Endocrinology 2000;141:4128–35.
[40] Ahima RS, Prabakaran D, Manzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382:250–2.
[41] Conway GS, Jacobs HS. Leptin a hormone of reproduction. Human Reproduction 1997;12:633–5.
[42] Yu WH, Kumura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic–pituitary function. Proceedings of the National Academy of Sciences of the United States of America 1997;94:1023–8.
[43] Ponzo OJ, Reynoso R, Rimoldi G, Rondina D, Szwarcfarb B, Carbone S, et al. Leptin stimulates the reproductive male axis in rats during sexual maturation by acting on hypothalamic excitatory amino acids. Experimental and Clinical Endocrinology and Diabetes 2005;13:135–8.
[44] Tena-Sempere M, Pinilla L, Gonzalez LC, Dieguez C, Casanueva FF, Aguilar E. Leptin inhibits testosterone secretion from adult rat testis in vitro. Journal of Endocrinology 1999;161:211–8.
[45] Blache D, Celi P, Blackberrv MA, Dynes RA, Martin GB. Decrease in voluntary feed intake and pulsatile luteinizing hormone secretion after intracerebroventricular infusion of recombinant bovine leptin in mature male sheep. Reproduction Fertility and Development 2000;12:373–81.