Leptin Is a Metabolic Signal for GnRH-LH/FSH Axis in Feed-restricted Ewes

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ABSTRACT : Two experiments were conducted to determine whether leptin is a metabolic signal for gonadotropin secretion in ewes. In the first experiment, twenty-eight cyclic Chal ewes were assigned randomly to an energy restricted, no leptin group (ERNL) (60% of maintenance; n = 14) and an energy normal, no leptin group (ENNL) (100% of maintenance; n = 14) for 71 days (6 estrous cycles). Estrus was synchronized with seven consecutive injections of $PGF_{2\alpha}$. Biweekly, body weight (BW) and body condition score (BCS) were determined and blood samples were collected to measure plasma leptin concentration. Blood samples were also taken to determine plasma progesterone concentration twice weekly. After each PG injection from the second injection to the end of experiment, four ewes were selected and blood samples were collected at 20 minutes and at hourly intervals for 3 h to detect plasma LH and FSH concentration. In the second experiment, after the ceasing of the estrous cycle caused by energy restriction, six acyclic ewes were selected and randomly allotted to two groups (n = 3) and received the following treatment for four days. Ewes in an energy restricted, leptin group (ERL) were fed with a ration which provided 60% of maintenance energy requirements and intravenously injected with 4 μg leptin/kg BW daily. Ewes in an energy excess, no leptin group (EENL) were fed with a ration that provided 180% (120%+60%) of maintenance energy requirements and intravenously injected with 1 ml saline daily. In both groups, blood samples were collected at 20 minutes and at hourly intervals for 3 h before feeding on d 0 and d 5, and for 3 h before and after injections as above on d 2 and d 4 to detect plasma LH and FSH concentration. In the first experiment, BW and BCS from the 2nd estrous cycle, and leptin from the 3rd estrous cycle to the end of the experiment significantly (p<0.05) decreased. In ERNL ewes, mean plasma concentrations of FSH significantly (p<0.01) decreased from the 4th estrous cycle to d 71 and LH pulsatile secretion was suppressed on d 71, so that, mean plasma concentrations of LH (p<0.05), LH pulse frequency (p<0.01) and LH pulse amplitude (p<0.05) significantly decreased. In the second experiment, injection of leptin significantly increased mean circulating concentrations of LH (p<0.05), LH pulse frequency (p<0.01), LH pulse amplitude (p<0.05) and mean circulating concentrations of FSH (p<0.01) and leptin (p<0.01). High energy intake significantly (p<0.05) stimulated pulsatile secretion of LH and leptin secretion (p<0.01), but non-significantly increased plasma FSH concentration. The results of this study indicate that leptin is a metabolic signal for the GnRH-LH/FSH axis in feed-restricted fat-tailed ewes. (**Key Words** : Leptin, Gonadotropin, Ewe, Metabolic Signal)

INTRODUCTION

Leptin, is a 16 kDa protein consisting of 146 amino acids which is synthesized primarily by adipose tissue and is secreted into the blood stream after cleavage of the 21 amino acids signal peptide (Zhang et al., 1994). Leptin regulates feed intake, energy balance, the neuroendocrine-axis and immunological processes in rodents, humans and ruminants (Houseknecht and Portocarero, 1998; Barb,

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1999; Cunningham et al., 1999). In the recent years, it has been established that leptin has stimulatory effect on gonadotropin secretion, in some of the mammalian species. Indeed, leptin is a metabolic signal for reproductive system (Barash et al., 1996; Cunningham et al., 1999; Barb et al., 1999a; Schneider et al., 2000). Intracerebroventricular (ICV) administration of leptin stimulated LH and FSH secretion in sheep (Fischer, 2001; Miller et al., 2001; Clarke, 2002). In vitro experiments also emphasize stimulatory effects of leptin on GnRH-LH/FSH axis (Barb et al., 1997, 1999b; Liou et al., 1997; Ridgway et al., 2000).

Among the nutritional factors, energy primarily affects reproductive processes (Dunn and Moss, 1992; Schillo, 1992; Wade and Schneider, 1992). Increase of energy intake in feed-restricted animals restored pulsatile LH secretion (Sisk and Bronson, 1986; Foster et al., 1989). It has been

Table 1. Experimental rations and prepared energy and nutrients

Diet	1[1]	2[2]	3[3]
Ingredients/nutrition			
Wheat straw (g/day)	10	260	600
Alfalfa (hay) (g/day)	50	50	50
Corn (grain) (g/day)	10	220	540
Corn gluten meal (g/day)	210	85	-
Bone meal (g/day)	1.34	0.47	2.5
Salt (g/day)	1.66	1.22	0.47
Magnesium oxide (g/day)	0.69	-	-
Vitamin and mineral supplement	3.50	3.50	3.50
Metabolizable energy (MJ/kg)	13.03	9.73	9.12
Crude protein (%)	42.00	13.72	7.18
Calcium (%)	0.52	0.24	0.22
Phosphorous (%)	0.52	0.24	0.22
Sodium (%)	0.45	0.21	0.11
Magnesium (%)	0.24	0.11	0.06
Dry mater intake (g/day)	287	620	1,197
Metabolizable energy intake (MJ/day)	3.74	6.03	10.90
Metabolizable protein intake (g/day)	56.00	55.37	57.95

[1] 60% of metabolizable energy and the other requirements at maintenance level.
[2] 100% of metabolizable energy and the other requirements at maintenance level.
[3] 180% of metabolizable energy and the other requirements at maintenance level.

indicated that there is a positive correlation between body energy content and leptin level (Bocquier et al., 1998; Houseknecht et al., 1998; Blache et al., 2000b; Delavaud et al., 2000; Towhidi et al., 2003; Cho et al., 2006). Leptin apparently is a humeral signal from adipose tissue for GnRH-LH/FSH axis in mammals. But mechanisms were not well elucidated in ruminants. The objective of this study was to investigate the role of leptin as a mediator of energy intake associated with secretion of gonadotropin in dietary energy-restricted ewes.

MATERIAL AND METHODS

Animals and location

Estrous cycles of 38 mature (2.5 years old) fat-tailed Chal ewes with moderate body condition (BCS = 2.5 to 3.5; CISRO, 1990) were synchronized with two 250 μg doses of Cloprestenol (an analogue of PGF2α, Nasr Ltd. Iran) on d -11 and 0 (d 0 = one day before the beginning of treatment). Estrous behavior was monitored in the presence of two teaser rams for 4 d following the second injection of Cloprestenol. The ewes were housed in single pen throughout the experiment. "Animal Science Research Institute of Karaj located at 51°2' West longitude and 35°48' North latitude".

Experimental design

Experiment 1: Twenty-eight ewes with synchronized estrous cycles were selected and randomly assigned to energy normal no leptin (ENNL) group (n = 14) or energy-restricted no leptin (ERNL) group (n = 14). The ewes in ERNL and ENNL were fed at 60% or 100% of maintenance energy requirements level by diet 1 and diet 2 (Table 1) for 71 days (or 6 estrous cycles). For induction of estrus, five consecutive injections of Cloprestenol were used on d 14, 28, 42, 56 and 70. Estrous behavior was monitored as above mentioned after each injection of Cloprestenol. Biweekly, body weight (BW) and body condition score (BCS) were determined on d -1, 13, 27, 41, 55 and 69.

Experiment 2: Few days after the experiment 1, six acyclic ewes (acyclic ewes, their estrous was not observed and plasma concentrations of progesterone (P₄) were lower than 1 ng/ml) from ERNL were selected and randomly allotted to two groups (n = 3) and received the following treatments from d 1 to d 5. All ewes in energy restricted leptin (ERL) group were fed with diet 1 that provided 60% of maintenance energy requirements and injected intravenously by human recombinant leptin (Mediagnostic Ltd., Germany) at 4 μg/kg BW every day. The ewes in energy excess no leptin (EENL) group were fed diet 3 (Table 1) that provided 180% (120%+60%) of maintenance energy requirements and intravenously injected by 1 ml physiologic saline every day.

Feeding

Gross energy and chemical composition of feedstuffs consisted of dry mater, crude protein, crude fiber, ether extract, total ash, NDF, ADF, calcium and phosphorous were analyzed in animal science research institute of Karaj. Diets were formulated based on AFRC (1995) (Table 1). During the course of the experiment, daily feed was weighed based on body weight and individually given to each ewe every morning. The ewes had free access to fresh water. Diet 1, 2 and 3 were consisted 60%, 100%, and 180% (120%+60%) of maintenance energy requirements, respectively. Other requirements were balanced at maintenance level.

Blood sampling

Experiment 1: Blood samples were taken (5 ml) by venepuncture through the jugular vein twice weekly to measure plasma progesterone (P₄) concentration. For consecutive blood sampling, four ewes from each group were selected and fitted under local xylocaine (2%) anesthesia with indwelling venous catheters in the jugular vein on d 0, 14, 28, 42, 56 and 70. When not in use, the catheters were filled with a solution of sterile heparinized saline (50 IU/ml). Blood samples were collected (3 ml) at 20 minutes and hourly intervals to detect plasma LH and
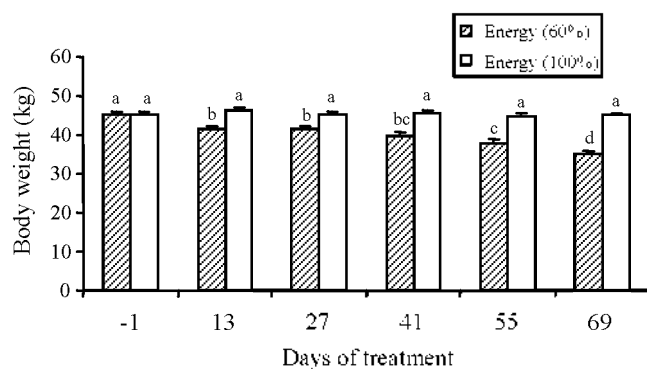
Figure 1. Mean (±SEM) body weight (kg) in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference (p<0.05).
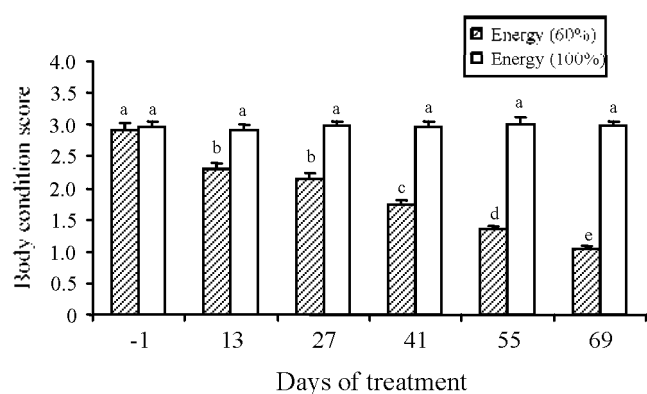

Figure 2. Mean (±SEM) body condition score in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference (p<0.05).

FSH concentration changes before feeding on day after fitting of catheter i.e. on day 1, 15, 29, 43, 57 and 71. Blood samples were also collected (5 ml) on above-mentioned days to detect plasma leptin concentration.

Experiment 2 : All ewes were fitted with indwelling venous catheters in the jugular vein on d -1 (two days before the beginning of treatments in the 2nd experiment). Blood samples were collected (3 ml) at 20 minutes and hourly intervals before feeding to detect plasma LH and FSH concentration changes on d 0 and d 5. Blood samples were also taken before and after injections on d 2 (2# and 2*, respectively) and d 4 (4# and 4*, respectively) as above. In addition, blood samples were collected (5 ml) on d 2 and d 4 (before and after injection), and d 0 and d 5 (before feeding) to measure plasma leptin and P_4 concentrations. The blood was centrifuged at 3,000 rpm for 20 minutes and the plasma separated and stored at -20°C.

Hormone assays

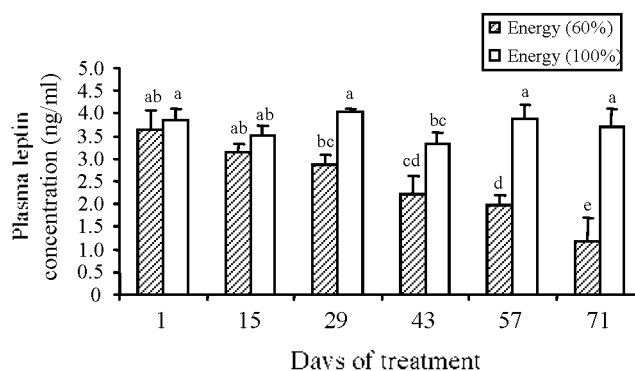Plasma concentrations of LH, FSH and leptin were determined in duplicated by RIA kit prepared for ovine

Figure 3. Mean (±SEM) plasma concentration of leptin in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference (p<0.05).

hormones and plasma P_4 concentrations were assayed by commercial RIA kit (Tabeshyar-noor Co., Iran). Assay sensitivity for LH, FSH. leptin and P_4 was 0.05 ng/ml. 0.04 ng/ml. 0.1 ng/ml and 0.05 ng/ml. respectively. The intra-assay coefficient of variation (CV) for LH, FSH, leptin and P_4 was 6.6%, 5.5%. 6.3% and 5.4. respectively. The inter-assay CV for LH, FSH. leptin and P_4 was 11.2%, 7.9%. 9.3% and 12.6%, respectively.

Statistical analyses

The LH pulses were identified by cluster algorithm procedure. Circulating concentrations of LH. FSH. leptin. P4 and frequency of LH pulses and amplitude of LH pulses were analyzed by the GLM models for repeated measures using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC). Sources of variation were treatment (main effect). time. and their interaction (treatment×time). Time was used as the repeated variable. and ewes within treatment were used as the subject. The least squares means procedure (PDIFF option) was used to compare means when significant F-value (p<0.05) was obtained.

RESULTS

Experiment 1

BW : Energy intake level (treatment). time and their interaction (treatment×time) had significant effect (p<0.01) on BW. Mean BW gradually decreased from d 14 to the end of experiment in ERNL ewes (p<0.01) (Figure 1).

BCS : Energy intake level, time and their interaction had significant effect (p<0.01) on BCS. Mean BCS gradually decreased from d 14 to the end of experiment in ERNL group (p<0.01) (Figure 2).

Leptin : Energy intake level, time and their interaction had significant effect (p<0.01) on plasma leptin concentration. Leptin concentration gradually decreased in

Table 2. Mean (±SEM) LH parameters in ewes that were fed with a ration that provided 60% or 100% of maintenance energy requirements for 71 days

Group / Days of treatment	ERNL group Amplitude (ng/ml)	Frequency (pulse/3 h)	Mean (ng/ml)	ENNL group Amplitude (ng/ml)	Frequency (pulse/3 h)	Mean (ng/ml)
1	$2.49^{ab}\pm0.44$	$1.25^{a}\pm0.25$	$1.25^{ab}\pm0.11$	$2.42^{ab}\pm0.19$	$1.25^{a}\pm0.25$	$1.21^{b}\pm0.08$
14	$2.34^{ab}\pm0.15$	$1.50^{a}\pm0.29$	$1.40^{ab}\pm0.06$	$1.86^{b}\pm0.08$	$1.50^{a}\pm0.29$	$1.22^{b}\pm0.10$
29	$2.28^{ab}\pm0.10$	$1.75^{a}\pm0.25$	$1.32^{ab}\pm0.09$	$2.05^{b}\pm0.13$	$1.50^{a}\pm0.29$	$1.45^{ab}\pm0.09$
43	$3.22^{a}\pm0.55$	$1.25^{a}\pm0.25$	$1.49^{ab}\pm0.14$	$2.04^{b}\pm0.17$	$1.25^{a}\pm0.25$	$1.27^{ab}\pm0.11$
57	$2.49^{ab}\pm0.40$	$1.00^{a}\pm0.00$	$1.54^{a}\pm0.08$	$2.24^{ab}\pm0.24$	$1.25^{a}\pm0.25$	$1.28^{ab}\pm0.80$
71	$2.55^{ab}\pm0.71$	$1.00^{a}\pm0.00$	$1.35^{ab}\pm0.11$	$0.00^{c}\pm0.00$	$0.00^{b}\pm0.00$	$0.93^{c}\pm0.05$

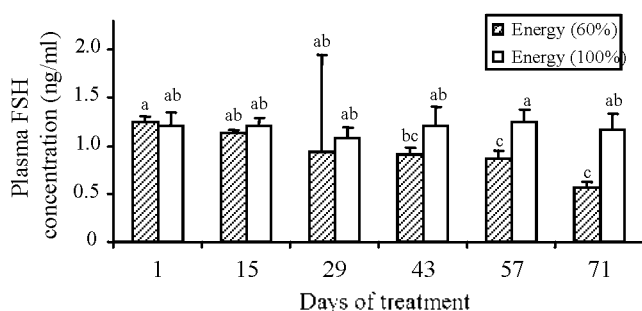Values without a common letter in each column and trait, have a significant difference ($p<0.05$).

Figure 4. Mean (±SEM) plasma concentration of FSH in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference ($p<0.05$).

ERNL group from d 29 compared with ENNL group, and from d 43 compared with itself ($p<0.01$) (Figure 3).

LH parameters : Energy intake level, time and their interaction had significant effect on LH pulse frequency ($p<0.01$), LH pulse amplitude ($p<0.05$) and mean concentration of LH ($p<0.05$). Overall plasma concentrations of LH, frequency of LH pulses (pulses/3 h) and amplitude of LH pulses were significantly less in ERNL group than in ENNL group on d 71 (Table 2).

FSH : Energy intake level, time and their interaction had significant effect ($p<0.05$) on mean concentrations of FSH. Overall plasma concentrations of FSH was significantly ($p<0.05$) less in RENL group than in ENNL group on d 57 (Figure 4).

Experiment 2

Leptin : Treatment, time and their interaction (treatment×time) had significant effect ($p<0.01$) on plasma leptin concentration (Figure 5). Recombinant human leptin markedly increased plasma concentrations of leptin in the ERL group after each injection ($p<0.01$). In the EENL group, leptin significantly ($p<0.01$) increased on d 4.

LH parameters : Treatment ($p<0.05$), time ($p<0.01$) and their interaction ($p<0.01$) had significant effect on LH pulse frequency and LH pulse amplitude and mean LH
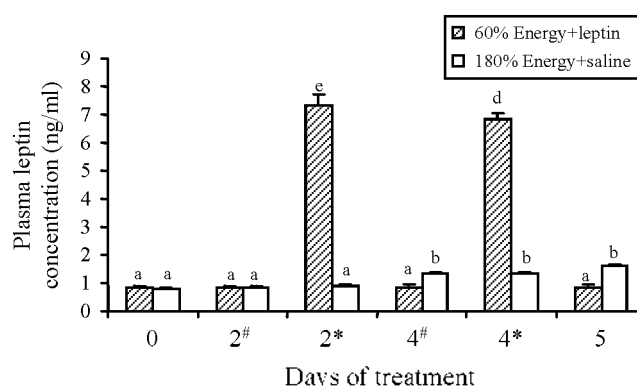

Figure 5. Mean (±SEM) plasma concentration of leptin in ewes that were treated by leptin and 60% of maintenance energy requirements (shaded bar) or saline and 180% (60%+120%) of maintenance energy requirements (open bar) in different times (#: before injection and *: after injection). Values without a common letter have a significant difference ($p<0.05$).

concentrations. In the ERL group, mean circulating concentrations of LH, and frequency and amplitude of the LH pulses were significantly ($p<0.05$) increased after each injection. In the EENL group, frequency and amplitude of the LH pulses were significantly ($p<0.05$) increased from d 4 after treatment. But, mean concentrations of LH did not differ throughout the experiment (Table 3).

FSH : Mean FSH concentrations in the ERL group were significantly ($p<0.05$) increased after each injection. In EENL group, circulatory concentration of FSH did not differ throughout the experiment (Figure 6).

DISCUSSION

In the first experiment, energy restriction resulted in decreased BW and BCS, but BW and BCS did not significantly change in ENNL group. BW and BCS had concomitant changes that were similar to the previous experiments (Gutierrez et al., 1987; Tatman et al., 1990). Diminished BW is usually caused by the decrease of body fat mass (Tatman et al., 1990; Schillo, 1992). Since there is a positive correlation between BCS and total body fat mass

Table 3. Mean (±SEM) LH parameters in ewes that were treated by leptin and 60% of maintenance energy requirements or saline and 180% (60%+120%) of maintenance energy requirements for five days

Group Days of treatment	ERL group			EENL group		
	Mean (ng/ml)	Frequency (pulse/3 h)	Amplitude (ng/ml)	Mean (ng/ml)	Frequency (pulse/3 h)	Amplitude (ng/ml)
0	$0.92^{ab}\pm0.07$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.98^{ab}\pm0.06$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
$2^{\#}$	$0.89^{a}\pm0.07$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.95^{ab}\pm0.09$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
2^{*}	$1.78^{c}\pm0.32$	$2.00^{d}\pm0.00$	$2.63^{cd}\pm0.19$	$1.06^{ab}\pm0.09$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
$4^{\#}$	$1.23^{abc}\pm0.28$	$1.33^{bcd}\pm0.33$	$3.03^{cde}\pm0.18$	$1.11^{ab}\pm0.19$	$0.67^{ab}\pm0.67$	$1.04^{ab}\pm1.04$
4^{*}	$1.77^{c}\pm0.32$	$2.00^{d}\pm0.00$	$3.42^{de}\pm0.29$	$1.23^{abc}\pm0.13$	$1.00^{bc}\pm0.58$	$1.73^{bc}\pm0.87$
5	$1.15^{ab}\pm0.22$	$1.00^{bc}\pm0.00$	$2.49^{cd}\pm0.27$	$1.02^{ab}\pm0.16$	$1.00^{bc}\pm0.00$	$3.93^{e}\pm0.30$

Values without a common letter in each column and trait, have a significant difference ($p<0.05$).
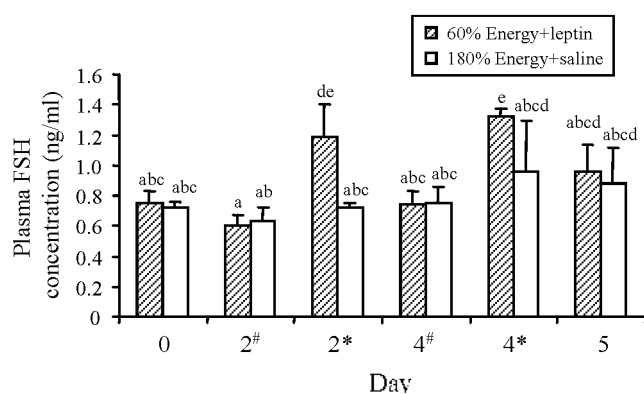$^{\#}$ Before injection. * After injection.

Figure 6. Mean (±SEM) plasma concentration of FSH in ewes that were treated by leptin and 60% of maintenance energy requirements (shaded bar) or saline and 180% (60%+120%) of maintenance energy requirements (open bar) in different times ($^{\#}$: before injection and *: after injection). Values without a common letter have a significant difference (p<0.05).

(Sanson et al., 1993), diminished BCS is probably caused by the decrease of body fat mass. However, body fat mass was not measure in the present experiment.

Decrease of energy intake level resulted in gradually decrease of leptin level during the course of experiment in ERNL ewes. These results were similar to the previous experiment in which ewe (Bocquier et al., 1998; Towhidi et al., 2003) and cow used (Chilliard et al., 1998a). Linear regression among the plasma leptin concentration, BW and BCS indicated that 0.25 and 0.48 of plasma leptin changes depended on BW and BCS changes, respectively. i.e. correlations between leptin level and BW was +0.5 (p<0.01), and correlation between leptin level and BCS was +0.7 (p<0.01). Considering strong relationship between BCS and back-fat thickness (Index of total body fat content), it seems that there is a relationship between blood leptin level and body fat mass. Blache et al. (2000b) have reported that there is stronger correlation between plasma leptin concentration and back-fat thickness and also back-fat thickness/BW ratio in ewe, ram and castrated ram. They have also demonstrated that 30% of blood leptin changes are caused

by back-fat thickness changes. Delavaud et al. (2000) have shown that there is a positive correlation between total body fat mass and plasma leptin level (r = +0.68, p<0.001), and between BCS and plasma leptin level (r = +0.72, p<0.001) in two and five years old ewes. In the recent study, the decrease in feeding level resulted in decrease to 56% in plasma leptin concentration. They concluded that 35% and 17% of blood leptin level changes depended on body fat mass and feeding, respectively. Chilliard et al. (1998a) reported that correlation between plasma leptin concentration and total body fat mass was +0.67 in ovariectomized ewes. In the late period of lactation in dairy cows, 37%, and in growing calves, 83% of blood leptin changes depend on BCS or body fat content, respectively (Ehrhardt et al., 2000). Marie et al. (2002) have shown that feeding of a ration that provided 90% of maintenance requirements for several months resulted in intense decrease of BW. BCS, white fat cells diameter in different areas and blood leptin concentration in Soay rams. Restriction of dietary energy to 0.4×maintenance for two weeks decreased leptin level in ewe (Bocquier et al., 1998). This is the first report on fat-tailed sheep which agrees with the previous studies, and shows a strong relationship between plasma leptin concentration and energy intake level. BW and specially BCS in fat-tailed Chal ewes.

Restriction of energy intake to 60% of maintenance resulted in a decrease of FSH level. Descending trend continued during consequent weeks. Kile et al. (1991) reported that long-term energy restriction decreased pituitary and plasma FSH concentration and βFSH-mRNA content in anterior pituitary in ewe. Expression of α- and β-FSH-mRNA and hypophyseal content of FSH decreased in cows under negative energy balance (Roche and Diskin, 1995). Blood FSH concentration in growth retarded female lambs was lower than those in control group (Foster et al., 1989). The results of the present study on fat-tailed ewes are similar to the previous studies. Restriction of dietary intake to 60% of maintenance in Chal ewes significantly decreased overall circulating LH concentration, frequency and amplitude of LH pulse after 71 days. In ewe, energy

restriction for three weeks resulted in either no change in LH pulse secretion (Abecia et al., 1995) or a modest decrease in LH pulse frequency (Rhind et al., 1989a; Rhind et al., 1989b). In the other study, it has been indicated that restriction of dietary to 0.4×maintenance had no effect on LH secretion in obese ewes, but suppressed pulsatile LH secretion in thin ewes with body fat content lower than 15% to 20% of their BW (Chilliard et al., 1998b). Consumption of a ration consisting 60% of maintenance energy requirements in ewes which was associated with severe decrease of BW and BCS resulted in a inhibition of pulsatile secretion of LH and ovulation (Tatman et al., 1990; Kile et al., 1991), and also plasma concentration of LH (Snyder et al., 1999) after a few months. Decrease of energy intake level caused delayed increase of pulsatile LH secretion and mean LH concentration in female growing lambs (Foster et al., 1989) and beef heifers (Yelich et al., 1996). In lactating cows under negative energy balance, α- and β-LH-mRNA content and LH content in anterior pituitary decreased (Roche and Diskin, 1995). The results of the present study confirm the previous experiments which indicated threshold effect of energy on LH secretion. Meanwhile, this experiment implicates severe decrease of body energy reservoirs is necessary for suppression of pulsatile LH secretion in fat-tailed ewes.

Inhibition of LH secretion is due to a suppressed release of GnRH from the hypothalamus (Kile et al., 1991; I'Anson et al., 2000). Feed restriction decreased GnRH pulse frequency, amplitudes, and the ability of low amplitude GnRH pulses to generate a concomitant LH pulse in ovariectomized lambs (I'Anson et al., 2000).

In the second experiment, peripherally injected leptin restored LH pulse secretion in long-term energy restricted fat-tailed ewes after 2 days. In ruminants, the previous studies have demonstrated the ability of chronic infused leptin to prevent food restriction-mediated decrease in the pulsatile LH secretion in sheep (Henry et al., 2001), cattle (Amstalden et al., 2000, 2003), heifers (Maciel et al., 2004). In the most studies, ICV administration of leptin was used, while leptin is a hormone primarily secreted by adipocytes into the blood stream (Houseknecht et al., 1998) and its plasma concentrations is index of body fat content and energy storage (Delavaud et al., 2000; Blache et al., 2000b). Hence, in this experiment, the effect of peripheral leptin injection on the hypothalamus-pituitary axis was investigated. Currently, the circumstance and mechanisms by which leptin can stimulate hypothalamic GnRH and/or adenohypophyseal LH secretion remain to be fully delineated, particularly in ruminants. However, there are some hypotheses.

Leptin has been detected in cerebroventricular fluid (Blache et al., 2000a,b), and its receptors found on the ventromedial and arcuate nuclei of hypothalamus that

secreting GnRH (Magni et al., 1999; Barb et al., 2000). NPY, and POMC (Finn et al., 1998). A study showed that leptin was able to affect higher areas of brain after the passage of throughout blood-brain barrier by specific receptors (Bjorbaek et al., 1998). Moreover, several reports have indicated that leptin can affect GnRH via neurotransmitters including NPY (Ahima et al., 1999; Lebrethon et al., 2000), POMC (Thornton et al., 1997), and CART (Christensen et al., 1997; Lebrethon et al., 2000). In vitro experiments have been also demonstrated that leptin induces hypothalamus explants to secrete GnRH (Yu et al., 1997; Barb et al., 1997; Barb et al., 1999a). Therefore, it has been suggested that leptin affect GnRH neurons either directly or via neurotransmitters.

Leptin receptors are distributed on anterior pituitary in sheep (Dyer et al., 1997; Magni et al., 1999; Iqbal et al., 2000). Moreover, leptin administration stimulates LH secretion from anterior pituitary in vitro (Barb et al., 1997; Barb et al., 2000; Barb et al., 2001; Yu et al., 1997; Liou et al., 1997; Ridgway et al., 2000). Thus, another hypothesis is direct effect of leptin on gonadotrope cells in the hypophysis to generate pulsatile secretion of LH.

Increase of energy intake level to 180% maintenance energy requirements result in an increase of leptin production and blood concentration after 4 days in ewes that their blood leptin levels had descended because of long-term energy restriction. Such results have been reported on ewe (Bocquier et al., 1998; Chilliard et al., 1998a), ram (Chilliard et al., 1998a; Blache et al., 2000b), and cow (Chilliard et al., 1998a). It has been shown an increase of leptin-mRNA expression and leptin production in adipose tissue a few hours after feeding in cattle and, also plasma leptin concentration during several days in ewes (Bocquier et al., 1998; Chilliard et al., 1998a).

High energy intake restored LH pulse secretion in long-term energy restricted ewes after 4 days. Moreover, LH pulse frequencies were enhanced from d 4 of experiment before the treatment and stayed at high level until one day after treatment period. LH pulse amplitude and LH pulse frequencies were lower in EENL ewes than in the ERL ewes immediately after 2nd injection. While, those were higher in EENL group than in ERL group on d 5. These results show that action of energy intake on hypothalamic-pituitary axis is more slowly than leptin's effect.

Chagas et al. (1999) have reported that high energy intake for five days resulted in elevated leptin level and LH pulse frequency in ram. In fed-restricted pigs, re-feeding for one week restored plasma leptin concentration and pulse frequency of LH to normal level (Whisnant and Harrell, 2001). In growth-retarded female lambs and rat (Sisk and Bronson, 1986; Foster et al., 1989) caused by food restriction, high energy level for four days caused to trigger pulsatile LH secretion. High feeding level also stimulated

LH secretion in ewes that to be bred in natural conditions (Rhind et al., 1985). High energy intake rapidly increased LH pulse frequencies without influencing LH pulse amplitudes in the women under low energy intake and high energy expenditure condition (Loucks and Verdun, 1998).

Results of the current study indicated that long-term energy restriction was necessary to suppress pulsatile LH secretion in ewes that had moderate energy storage in body, but increase of energy intake level for several days was enough to restore LH pulses. Considering to observed relationships between blood leptin level and LH pulse secretion, and also stimulatory effect of leptin administration on LH secretion, it seems necessary to spend a period of time of dietary energy restriction, in order that blood leptin level descends to a putative inhibitory threshold which suppresses pulsatile secretion of LH. The length of this period is depending on primary leptin level i.e. body fat content. By contrast, a short-term high energy intake raised plasma leptin level which could overcome this inhibitory threshold, and subsequently stimulated the hypothalamic-pituitary-gonadal axis. Contrary to feed restricted ruminants, administration of leptin failed to alter LH secretion in well nourished intact ewes (Towhidi et al., 2004), ovariectomized ewes (Hanry et al., 1999), lambs (Morrison et al., 2001) and heifers (Maciel et al., 2004). In a recent report, leptin treatment stimulated basal and GnRH-mediated LH secretion from pituitary explants from fasted, but not control fed cows, which having no effect on GnRH release from hypothalamic explants from either group of cows (Amestalden et al., 2003). In an unpublished study, we observed that peripheral injection of leptin could not change the pattern of LH secretion in well-fed ewes. Thus, metabolic condition appears to be a primary determinant of the hypothalamic-pituitary response to leptin in ruminants. Importantly, one of the primary factors associated with an increase in the number of leptin receptors in the ventromedial hypothalamus of ewes is malnutrition (Dyer et al., 1997). It has been suggested that leptin may only stimulate LH secretion during nutritional stress in ruminants. Hence, both fasting and chronic, severe dietary energy restriction appear to hypersensitized the hypothalamic-hypophyseal axis to leptin.

Intravenously injected leptin (4 µg/kg BW) resulted in an increase of circulating FSH concentration after each injection in dietary energy-restricted ewes. It has been previously indicated in ob/ob male and female mice that administration of leptin stimulated FSH secretion (Barash et al., 1996). Moreover, leptin treatment induced secretion of FSH from pituitary explants in castrated bull and mouse (Liou et al., 1997), and female rat (Liou et al., 1997). The results of the current experiment confirm the previous studies. However, this is the first report on stimulatory effect of peripherally injected leptin on FSH secretion in

dietary energy restricted fat-tailed ewes. Presumed mechanisms by which leptin affect GnRH-FSH axis are similar to GnRH-LH axis.

Increase of energy intake level to 180% maintenance energy requirements failed to alter significantly plasma FSH concentration, although a progressive trend was detected during the late experimental period. Secretion of FSH in the growth-retarded ewes, caused by chronic fed deprivation was induced by a 14 days period of high-energy intake (Foster et al., 1989). The reason of this difference probably is to be shorter re-feeding period in the current study. However, there are some reports which agree with our data (Ritar and Adams, 1988; Rhind et al., 1989a; Downing et al., 1995). Contrary to FSH, secretion of LH was markedly increased by high energy intake in this experiment. The reason of this phenomenon is probably to exist a strong mechanism controlling FSH secretion compared with LH. e.g. negative feedback of ovarian steroids and inhibin (Brooks et al., 1999). On the other hand, LH secretion responded to feeding level and BCS under a definite threshold (Tatman et al., 1990), i.e. LH pulse secretion is existing while feeding level and BCS to be higher than a critical level. It seems that there is not such mechanism for FSH secretion.

In this study, progressive trend of FSH during the late experiment was concordant with elevated leptin level in high energy intake ewes. Considering to stimulatory effect of leptin injection on FSH, it appears that action of dietary energy intake on FSH secretion is mediated by leptin as LH. but mechanisms involved in FSH regulation are more complex.

In the present experiment, the response of FSH to leptin administration resembled to LH. Similarly, ICV (Carro et al., 1997) or peripheral (Ahima et al., 1996; Barash et al., 1996) administration of leptin in rodents resulted in alteration of pituitary endocrine function and synchronous stimulation of FSH and LH secretion. However, whether these effects directly act on hypophysotropic cells of hypothalamus or endocrine cells of anterior pituitary is still ambiguous, particularly in ruminants.

IMPLICATIONS

The current results suggest that energy acts on LH secretion under the threshold condition, but gradually affects FSH secretion in fat-tailed Chal ewes. Leptin level and BCS have a positive correlation in these ewes, similar to the other animals. Pulsatile LH secretion was suppressed when the blood leptin level descend to a putative inhibitory threshold. High energy intake and peripheral leptin injection could restore gonadotropins secretion to normal level in energy-restricted ewes. Thus, it can be concluded that leptin

is a metabolic signal for GnRH-LH/FSH axis in feed-restricted ewes.

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