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The Effects of Energy on the Gonadotrophins Secretion are Mediated by Leptin in Ewes

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Abstract: Two experiments were conducted to investigate the ability of leptin to mediate the effects of energy on the gonadotrophins secretion. In the first experiment, twenty-eight cyclic Chal ewes were assigned randomly to dietary energy restricted (60% of maintenance; n = 14) and control (100% of maintenance; n = 14) groups for 71 days (6 estrous cycles). Estrus was synchronized with seven consecutive injections of PGF2a. 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 and from second injection to the end of experiment, four ewes were selected and blood samples were collected at 20 min and hourly intervals for 3 h to detect plasma LH and FSH concentration. In the 2nd experiment, after the ceasing of estrous cycle caused by energy restriction, six acyclic ewes were selected and randomly alloted to two groups (n = 3) and received the following treatment for four days. All ewes in group I were fed with a ration which provided 60% of maintenance energy requirements and intravenously injected with 4 μg leptin kg^{-1} BW in every day. All ewes in group II were fed with a ration that provided 180% (120+60%) of maintenance energy requirements and intravenously injected with 1 mL saline in every day. In both groups, blood samples were collected at 20 min and hourly intervals for 3 h before feeding on day 0 and day 5 and for 3 h before and after injections as above on day 2 and day 4 to detect plasma LH and FSH concentration. In the first experiment, BW and BCS from the second estrous cycle and leptin from the third estrous cycle to end of experiment significantly ($p<0.05$) decreased. In the dietary energy restricted ewes, mean plasma concentrations of FSH significantly ($p<0.01$) decreased from fourth estrous cycle to day 71 and LH pulsatile secretion suppressed on day 71, so that, mean plasma concentrations of LH ($p<0.05$) and 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 ($p<0.01$) and 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 increased non-significantly plasma FSH concentration. The results of this study imply that the effects of energy on the gonadotrophins secretion are mediated by leptin in ewe.

Key words: Energy, leptin, gonadotrophin, ewe

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, 1999; Cunningham *et al*., 1999). In the recent years, it has been established that leptin has stimulatory effect on gonadotrophin 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)

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administration of leptin (Miller *et al*., 2001; Henry *et al*., 2001; Clarke, 2001) stimulated LH and FSH secretion in sheep. *In vitro* experiments also emphasize stimulatory effects of leptin on GnRH-LH/FSH axis have been also shown by *in vitro* experiments (Liou *et al*., 1997; Barb *et al*., 1997, 1999b; Ridgway *et al*., 2000).

Among the nutritional factors, energy primarily affects reproductive processes (Dunn and Moss, 1992; Wade and Schneider, 1992; Schillo, 1992). Increase of energy intake in fed-restricted animals restored pulsatile LH secretion (Sisk and Bronson, 1986; Foster *et al*., 1989). It has been indicated that there is a positive correlation between body energy content and leptin level (Houseknecht *et al*., 1998; Bocquier *et al*., 1998; Blache *et al*., 2000b; Delavaud *et al*., 2000). Therefore, it is possible that energy effect on some of the reproductive processes is mediated by leptin, but mechanism was not well understood. The objective of this study was to determine the effect of peripheral leptin injection (4 μg kg^{-1} BW) and increased energy level intake (180%) on gonadotropin secretion in dietary energy-restricted ewes.

MATERIALS 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 Cloprostenol (an analogue of PGF_2a, Nasr Ltd. Iran) on day -11 and 0 (day 0 = one day before the beginning of treatment). Estrous behavior was monitored in the presence of two teaser rams for 4 day following the 2nd injection of Cloprostenol. The ewes were housed in single pen throughout the experiment, Animal Science Research Institute of Karaj (located at 51°.2' W, 35°.48' N latitude).

Experimental design

Experiment 1: Twenty-eight ewes with synchronized estrous cycles were selected and randomly assigned to control (n = 14) or dietary energy-restricted (treated; n = 14) groups. The ewes were fed at 60% (treatment) or 100% (control) of maintenance energy requirements level by diet 1 and diet 2 (Table 1) for 71 days (or 6 estrous cycles). To remain estrous synchronization, five consecutive injections of Cloprestenol were used on day 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.

Table 1: Experimental rations and prepared energy and nutrients

	Diet		
Ingredients/nutrition	1[1]	2[2]	3[3]
Wheat straw (g day^{-1})	10	260	600
Alfalfa (hay) (g day^{-1})	50	50	50
Corn (grain) (g day^{-1})	10	220	540
Corn gluten meal (g day^{-1})	210	85	-
Bone meal (g day^{-1})	1.34	0.47	2.5
Salt (g day^{-1})	1.66	1.22	0.47
Magnesium oxide (g day^{-1})	0.69	-	-
Vitamin and mineral supplement	3.50	3.50	3.50
Metabolizable energy (Mj kg^{-1})	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^{-1})	287	620	1197
Metabolizable energy intake (Mj day^{-1})	3.74	6.03	10.90
Metabolizable protein intake (g day^{-1})	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

Experiment 2: Few days after the experiment 1, six acyclic ewes (acyclic ewes, their estrous was not observed and plasma concentrations of progesterone (P_4) were lower than 1 ng mL^{-1}) from energy-restricted group were selected and randomly allotted to two groups (n = 3) and received the following treatments from day 1 to 5. All ewes in Group I (leptin 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^{-1} BW every day. The ewes in group II (high energy group) were fed diet 3 (Table 1) that provided 180% (120+60%) of maintenance energy requirements and intravenously injected by 1 mL physiologic serum (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 analysed 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 (P_4) 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 day 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^{-1}). Blood samples were collected (3 mL) at 20 min and hourly intervals to detect plasma LH and 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 day 1 (two days before the beginning of treatments in the 2nd experiment). Blood samples were collected (3 mL) at 20 min and hourly intervals before feeding to detect plasma LH and FSH concentration changes on day 0 and day 5. Blood samples were also taken before and after injections on day 2 ($2^{\#}$ and 2*, respectively) and day 4 ($4^{\#}$ and 4*, respectively) as above. In addition, blood samples were collected (5 mL) on day 2 and day 4 (before and after injection) and day 0 and day 5 (before feeding) to measure plasma leptin and P_4 concentrations. The blood was centrifuged at 3000 rpm for 20 min 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 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, 0.04, 0.1 and 0.05 ng mL^{-1}, respectively. The intraassay 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, P_4 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) (SAS Institute Inc, 1996). 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 day 14 to the end of experiment in energy-restricted ewes ($p<0.01$) (Fig. 1).

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

Leptin: Energy intake level, time and their interaction had significant effect ($p<0.01$) on plasma leptin concentration. Leptin concentration gradually decreased in energy-restricted ewes from d 29 compared with control and from day 43 compared with itself ($p<0.01$) (Fig. 3).

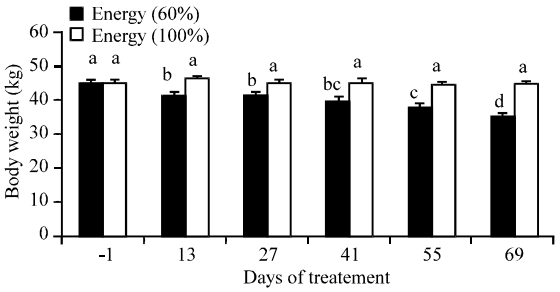

Fig. 1: Mean±SEM body weight (kg) in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference ($p<0.05$)

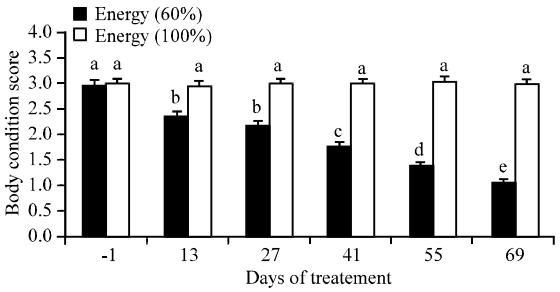

Fig. 2: Mean±SEM body condition score in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference ($p<0.05$)

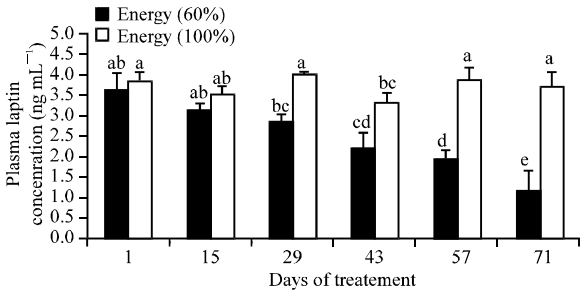

Fig. 3: Mean±SEM plasma concentration of leptin in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference ($p<0.05$)

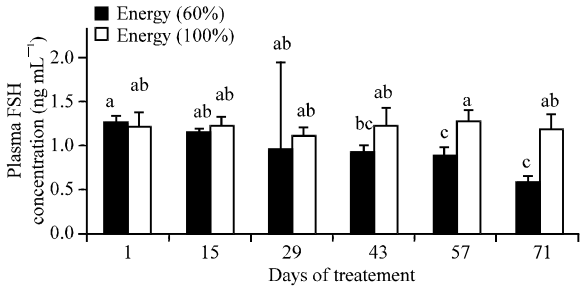

Fig. 4: Mean±SEM plasma concentration of FSH in ewes that were fed with a ration that provided 60% (shaded bar) or 100% (open bar) of maintenance energy requirements for 71 days. Values without a common letter have a significant difference ($p<0.05$)

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^{-1}) and amplitude of LH pulses were significantly less in treated group than in control on day 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 treated group than in control on day 57 (Fig. 4).

Experiment 2

Leptin: Treatment, time and their interaction (treatment * time) had significant effect ($p<0.01$) on plasma leptin concentration (Fig. 5). Recombinant human leptin markedly increased plasma concentrations of leptin in

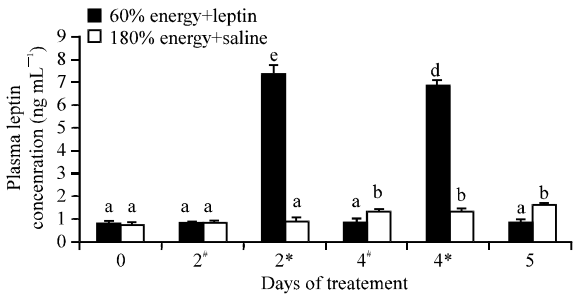

Fig. 5: Mean±SEM plasma concentration of leptin in ewes that were treated by leptin and 60% of maintenance energy requirements (shaded bar) or saline and 180% (60+120%) of maintenance energy requirements (open bar) in different times (#: before injection and *: after injection). Values without a common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

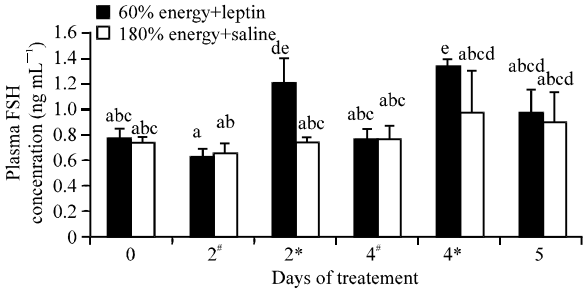

Fig. 6: Mean±SEM plasma concentration of FSH in ewes that were treated by leptin and 60% of maintenance energy requirements (shaded bar) or saline and 180% (60+120%) of maintenance energy requirements (open bar) in different times (#: before injection and *: after injection). Values without a common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

the leptin group after each injection ($p<0.01$). In high energy group, leptin significantly ($p<0.01$) increased on day 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 concentrations. In leptin group, mean circulating concentrations of LH and frequency and amplitude of the LH pulses were significantly ($p<0.05$) increased after each injection. In high energy group, frequency and amplitude of the LH pulses were significantly ($p<0.05$) increased from day 4 after treatment. But, mean concentrations of LH did not differ throughout the experiment (Table 3).

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					
	Fed-restricted			Well-fed		
Days of treatments	Mean (ng mL^{-1})	Frequency (pulse 3h^{-1})	Amplitude (ng mL^{-1})	Mean (ng mL^{-1})	Frequency (pulse 3h^{-1})	Amplitude (ng mL^{-1})
1	1.21±0.08^{b}	1.25±0.25^{a}	2.42±0.19^{ab}	1.25±0.11^{ab}	1.25±0.25^{a}	2.49±0.44^{ab}
14	1.22±0.10^{b}	1.50±0.29^{a}	1.86±0.08^{b}	1.40±0.06^{ab}	1.50±0.29^{ab}	2.34±0.15^{ab}
29	1.45±0.09^{ab}	1.50±0.29^{a}	2.05±0.13^{b}	1.32±0.09^{ab}	1.75±0.25^{a}	2.28±0.10^{ab}
43	1.27±0.11^{ab}	1.25±0.25^{a}	2.04±0.17^{b}	1.49±0.14^{ab}	1.25±0.25^{a}	3.22±0.55^{a}
57	1.28±0.80^{ab}	1.25±0.25^{a}	2.24±0.24^{ab}	1.54±0.08^{a}	1.00±0.00^{a}	2.49±0.40^{ab}
71	0.93±0.05^{c}	0.00±0.00^{b}	0.00±0.00^{c}	1.35±0.11^{ab}	1.00±0.00^{a}	2.55±0.71^{ab}

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

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					
	60% energy+leptin			(60+120%) energy+saline		
Days of treatments	Mean (ng mL^{-1})	Frequency (pulse 3h^{-1})	Amplitude (ng mL^{-1})	Mean (ng mL^{-1})	Frequency (pulse 3h^{-1})	Amplitude (ng mL^{-1})
0	0.92±0.07^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.98±0.06^{ab}	0.00±0.00^{a}	0.00±0.00^{a}
2$^{\#}$	0.89±0.07^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.95±0.09^{ab}	0.00±0.00^{a}	0.00±0.00^{a}
2*	1.78±0.32^{c}	2.00±0.00^{d}	2.63±0.19^{cd}	1.06±0.09^{ab}	0.00±0.00^{a}	0.00±0.00^{a}
4$^{\#}$	1.23±0.28^{a-c}	1.33±0.33^{b-d}	3.03±0.18^{c-e}	1.11±0.19^{ab}	0.67±0.67^{ab}	1.04±1.04^{ab}
4*	1.77±0.32^{c}	2.00±0.00^{d}	3.42±0.29^{de}	1.23±0.13^{a-c}	1.00±0.58^{bc}	1.73±0.87^{bc}
5	1.15±0.22^{ab}	1.00±0.00^{bc}	2.49±0.27^{cd}	1.02±0.16^{ab}	1.00±0.00^{bc}	3.93±0.30^{e}

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

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

DISCUSSION

In the first experiment, energy restriction resulted in decreased BW and BCS, but BW and BCS did not significantly change in control. 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 (Sanson *et al*., 1993), diminished BCS is probably caused by the decrease of body fat mass. However, body fat mass did not measure in the present experiment.

Decrease of energy intake level resulted in gradually decrease of leptin level during the course of experiment in treated ewes. These results were similar to the previous experiment in which ewe (Bocquier *et al*., 1998) 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 to 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 more strong 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 study 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 β-FSH-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 during 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,b). 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, a- and ßLH-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 2nd 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*., 1997,1999a). 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, 2000, 2001; Yu *et al*., 1997; Liou *et al*., 1997; Ridgway *et al*., 2000). Thus, another hypothesis is direct effect of leptin on gonadotroph 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 day 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 high energy fed ewes than in the leptin-treated ewes immediately after 2nd injection. While, those were higher in high energy group than in leptin group on day 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 (Foster *et al*., 1989; Sisk and Bronson, 1986) 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 long of this period is depending on primary leptin level i.e., body fat content. By contraries, 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, chronic administration of leptin failed to alter LH secretion in well nourished ovariectomized ewes (Henry *et al*., 1999), lambs (Morrison *et al*., 2001) and heifers (Maciel *et al*., 2004). In a recent study, 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 (Amstalden *et al*., 2003). In a 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, sever dietary energy restriction appear to hypersensitize the hypothalamic-hypophyseal axis to leptin.

Intravenously injected leptin (4 μg kg^{-1} BW) resulted in an increase of circulating FSH concentration after each injection in dietary energy-restricted ewes. It has been earlier 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 and female rat (Liou *et al*., 1997). The results of the current experiment confirm the previous studies. However, this is the first study 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 are agreement with our data (Ritar and Adams, 1988; Rhind *et al*., 1989a; Downing and Scaramuzzi, 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 inhibit (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.

CONCLUSION

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 gonadotrophins secretion to normal level in energy-restricted ewes. Thus, we can be concluded that the effects of energy on gonadotrophins secretion are mediated by leptin.

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