Endocrine, *vol. 17, no. 3, 175–184, April 2002* 0969–711X/02/17:175–184/$12.50

Pulsatile Leptin Secretion Is Independent of Luteinizing Hormone Secretion in Prepubertal Sheep

Sergio E. Recabarren,[1] Alejandro Lobos,[1] Cecilia Vilches,[1] Paulina Muñoz,[1] and Teresa Sir-Petermann[2]

[1]Laboratory of Animal Physiology and Endocrinology, Faculty of Veterinary Medicine, University of Concepción, Campus Chillán, Chile; and [2]Laboratory of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Universidad De Chile, Santiago, Chile

Many studies have suggested that leptin modulates the gonadal axis. A synchronicity of luteinizing hormone (LH) and leptin has been described in humans, suggesting that leptin may modulate the episodic secretion of LH. The objective of this study was to establish whether episodic leptin secretion depends on the episodic LH secretion in prepubertal sheep. We used two different approaches. The first consisted of blocking the release of LH using a long-acting LH-releasing hormone (LHRH) agonist and analyzing the episodic LH and leptin secretions. The second method stimulated the pituitary gland with pulses of LHRH and again LH and leptin secretions were analyzed. Spring-born 20-wk-old Suffolk ewe lambs (n = 5) received intramuscularly a long-acting LHRH agonist (Decapeptyl®). Treatment was repeated at 24 and 28 wk of age. Control lambs (n = 6) received the vehicle of Decapeptyl. Diurnal and nocturnal pulsatilities of LH and leptin were studied at 20 (before Decapeptyl injection), 26, and 30 wk. Blood samples were taken at 10-min intervals for 6 h, beginning at 10:00 AM (diurnal sampling) and at 10:00 PM (nocturnal sampling). In all samples, LH and leptin were measured by radioimmunoassay, and pulsatile hormone secretion characteristics were assessed by the CLUSTER program. To characterize further the synchronicity between LH and leptin pulses, LHRH (10 ng/kg body wt) was injected at 60-min intervals, six times, to another five 30-wk-old ewe lambs, for the same time period as the pulsatility study. In the control group, LH secretion did not change between lambs of 20 and 30 wk of age. In LHRH agonist–treated lambs, LH secretion diminished from 20 to 30 wk of age and was lower than in control lambs at 26 and 30 wk of age ($p < 0.05$). The transversal mean (ng/[mL·6 h]) of leptin concentrations was different between control lambs of 20 wk of age and 26 and 30 wk of age ($p < 0.01$). Contrary to the findings in LH secretion, in LHRH agonist–treated lambs, mean plasma leptin concentrations did not decrease. Furthermore, the mean diurnal and nocturnal leptin concentrations and the pulse amplitude were higher at 26 and 30 wk than at 20 wk in LHRH agonist–treated lambs ($p < 0.05$). There were no differences between diurnal and nocturnal parameters of leptin secretion in both groups. There was no synchronicity between LH and leptin pulses. LHRH pulses significantly increased plasma LH concentrations, producing discernible LH pulses; however, leptin amplitude and leptin pulse frequency were not modified by the exogenous LHRH pulses, exhibiting no coincidence with LH pulses. The data suggest that pulsatile leptin secretion is independent of LH secretion in ewe lambs.

Key Words: Luteinizing hormone; leptin; ewe lamb; copulsatility.

Introduction

The initiation of puberty in female sheep depends on a complex interplay between external and internal determinants whose final goal is the hypothalamus *(1)*. The result of this neuroendocrine integration in the hypothalamus is the promotion of pulsatile luteinizing hormone–releasing hormone (LHRH) secretion, with an evident increase in pulse frequency. The increase in LHRH/luteinizing hormone (LH) secretion may be modulated by different metabolic signals of blood origin, which inform the LHRH pulse generator about the metabolic status of the growing female. One of these signals may be leptin.

Leptin is a hormone produced mainly by adipocytes that has been postulated as a permissive metabolic signal in different species, linking the energetic storage necessary for a successful pregnancy and the increase in LHRH secretion for the onset of puberty *(2,3)*. In female rats, a minimal plasma leptin concentration of 700 pg/mL seems to be a threshold level for sexual maturation to progress *(4)*. On the other hand, central administration of leptin in female rats under

Received December 4, 2001; Revised March 13, 2002; Accepted March 13, 2002.

Author to whom all correspondence and reprint requests should be addressed: Prof. Sergio E. Recabarren, Universidad de Concepción, Casilla 537, Chillán, Chile. E-mail: srecabar@mail.udec.cl

Table 1
Characteristics of Diurnal and Nocturnal Pulsatile LH Secretions in Control and LHRH Agonist–Treated Ewe Lambs 20, 26, and 30 wk of Age

	Mean (ng/[mL·6 h])	No. of significant peaks/6 h	Amplitude of pulses (ng/mL)	Nadir (ng/mL)
Control lambs				
20 wk				
Diurnal	0.35 ± 0.07	4.5 ± 0.8	0.65 ± 0.15	0.22 ± 0.08
Nocturnal	0.43 ± 0.06	4.3 ± 0.5	0.71 ± 0.12	0.27 ± 0.03
26 wk				
Diurnal	0.42 ± 0.07	4.2 ± 0.6	0.91 ± 0.22^{x}	0.25 ± 0.07
Nocturnal	0.41 ± 0.09	3.0 ± 0.4	1.09 ± 0.27^{x}	0.16 ± 0.04
30 wk				
Diurnal	0.44 ± 0.08^{a}	4.7 ± 0.3	0.82 ± 0.16^{x}	0.33 ± 0.07
Nocturnal	0.38 ± 0.04^{a}	4.8 ± 0.3	0.66 ± 0.14^{x}	0.28 ± 0.07
LHRH Agonist–treated lambs				
20 wk				
Diurnal	0.34 ± 0.14^{c}	4.4 ± 0.6	0.48 ± 0.22^{y}	0.24 ± 0.09
Nocturnal	0.27 ± 0.05	4.6 ± 0.5	0.34 ± 0.05^{y}	0.18 ± 0.06
26 wk				
Diurnal	0.23 ± 0.04	6.8 ± 0.4	0.33 ± 0.06^{y}	0.18 ± 0.04
Nocturnal	0.23 ± 0.05	5.8 ± 0.7	0.32 ± 0.06^{y}	0.18 ± 0.04
30 wk				
Diurnal	0.13 ± 0.01^{b,d}	5.4 ± 0.6	0.21 ± 0.01^{y}	0.10 ± 0.0
Nocturnal	0.13 ± 0.01^{b}	4.6 ± 1.3	0.20 ± 0.03^{y}	0.10 ± 0.0

$^{a,b}p < 0.01$.
$^{c,d}p < 0.05$.
$^{x,y}p < 0.05$.

severe food restriction allows the initiation of puberty defined by the vaginal opening, despite the body weight loss and increase in energy waste resulting from the effect of leptin on metabolism *(4)*. In normal growing female rats, the effect of leptin was compared with that obtained in female rats fed ad libitum or under food restriction. The onset of puberty and other indices of sexual maturation were not different in leptin-treated rats compared with control rats, even though the body weight was lower, because food consumption was 80% lower than in control female rats, whereas food-restricted rats exhibited a delay in the onset of puberty *(5)*. In female mice, the administration of leptin during normal prepubertal development stimulated an early onset of puberty, despite a lower body weight than in untreated control females *(6)*.

In humans, it has been observed that girls show higher levels of circulating leptin than boys *(7,8)*. Differences in the concentrations remain even after correction of values by body weight index and body fat mass *(9,10)*. After puberty, leptin concentrations continue to increase in pubertal girls, whereas they decrease in boys. This suggests a dimorphism in the leptin secretion that could be related to the gonadal steroid production. In a recent study by Palmert et al. *(11)*, in boys with central precocious puberty treated with an LHRH analog to suppress the gonadotropin and gonadal steroid secretion, it was concluded that testosterone reduces plasma leptin concentrations. In addition, the nocturnal rhythm of leptin persisted even with the suppression of the gonadal axis, which suggested an independence between the diurnal rhythm in leptin concentrations and the state of the reproductive axis.

These findings raise the possibility that the secretions of leptin and LH are independent. To analyze further the relationship between LH and leptin secretions, we studied the episodic fluctuations of circulating LH and leptin in normal growing ewe lambs under two different interventions. One consisted of blocking the release of LH using a long-acting LHRH agonist *(12)* and analyzing the diurnal and nocturnal episodic LH and leptin secretions at 20, 26, and 30 wk of age. Furthermore, the synchronicity between LH and leptin pulses was determined. The other approach was to stimulate the pituitary gland with pulses of LHRH and again analyze LH and leptin secretions.

Results

Study of Pulsatile LH and Leptin Secretions

Table 1 shows the characteristics of the diurnal and nocturnal pulsatile LH secretions in control and LHRH agonist-treated ewe lambs. In control lambs, mean plasma LH concentrations, pulse frequency, pulse amplitude, and nadir concentrations did not change between 20 and 30 wk of

Table 2
Characteristics of Diurnal and Nocturnal Pulsatile Leptin Secretions in Control and LHRH Agonist–Treated Ewe Lambs 20, 26, and 30 wk of Age

	Mean (ng/[mL·6 h])	No. of significant peaks/6 h	Amplitude of pulses (ng/mL)	Nadir (ng/mL)
Control lambs				
20 wk				
Diurnal	1.55 ± 0.06[a]	5.8 ± 0.5	2.00 ± 0.18[e]	1.18 ± 0.05
Nocturnal	1.51 ± 0.08[a]	5.6 ± 0.5	1.83 ± 0.07[e]	1.12 ± 0.06
26 wk				
Diurnal	2.18 ± 0.12[b,x]	5.8 ± 0.4	2.63 ± 0.15[f]	1.82 ± 0.12
Nocturnal	1.98 ± 0.13[b]	4.6 ± 0.7	2.45 ± 0.19[f]	1.42 ± 0.15
30 wk				
Diurnal	2.01 ± 0.25[b]	6.2 ± 0.5	2.40 ± 0.28[f]	1.63 ± 0.18
Nocturnal	1.86 ± 0.30[b]	6.0 ± 0.4	2.20 ± 0.32[f]	1.48 ± 0.29
LHRH Agonist–treated lambs				
20 wk				
Diurnal	1.53 ± 0.06[c]	5.4 ± 0.4	1.83 ± 0.10[g]	1.18 ± 0.05
Nocturnal	1.47 ± 0.06[c]	6.0 ± 0.5	1.80 ± 0.07[g]	1.10 ± 0.04
26 wk				
Diurnal	1.80 ± 0.04[d,y]	4.8 ± 0.4	2.25 ± 0.14[h]	1.48 ± 0.09
Nocturnal	1.75 ± 0.05[d]	5.0 ± 0.3	2.24 ± 0.09[h]	1.44 ± 0.09
30 wk				
Diurnal	1.93 ± 0.12[d]	5.8 ± 0.5	2.40 ± 0.14[h]	1.66 ± 0.14
Nocturnal	1.93 ± 0.14[d]	6.0 ± 0.7	2.30 ± 0.14[h]	1.60 ± 0.21

[a,b] $p < 0.01$.
[c,d] $p < 0.01$.
[e,f] $p < 0.01$.
[g,h] $p < 0.05$.
[x,y] $p < 0.05$.

age. The LHRH agonist significantly diminished the mean plasma LH concentrations, the mean pulse amplitude, and the mean of nadir concentrations in the ewe lambs between 20 and 30 wk of age, with no change in pulse frequency. At 30 wk of age, mean plasma LH concentrations, mean amplitude, and mean nadir were significantly lower in the LHRH agonist–treated group than in the control group. There was no difference between day and night LH secretions in both groups.

Table 2 summarizes the characteristics of the diurnal and nocturnal pulsatile leptin secretions in control and LHRH agonist–treated ewe lambs. In the control group, leptin concentrations increased significantly between 20 and 30 wk of age ($p < 0.01$). Contrary to what was found in LH secretion, leptin secretion did not decrease in the LHRH agonist–treated group. The mean diurnal and nocturnal leptin concentrations and the pulse amplitude were higher at 30 wk than at 20 wk ($p < 0.05$). However, diurnal leptin concentrations and leptin pulse amplitude were lower in the LHRH agonist group than in the control group at 26 wk. There were no differences between diurnal and nocturnal parameters of leptin secretion in both groups.

Diurnal and nocturnal plasma LH and leptin profiles are shown in Figs. 1 and 2 for the control group and in Figs. 3 and 4 for the LHRH agonist–treated group. There was no synchronicity between LH and leptin pulses according to the ANCOPULS analysis in the control group.

Effect of LHRH Pulses on LH and Leptin Secretion

Figure 5 displays diurnal LH and leptin profiles in 30-wk-old lambs treated with pulses of LHRH. Each LHRH pulse produced a significant increase in plasma LH concentrations, imposing an artificial LH pulse frequency with no alteration in leptin pulse frequency or amplitude. Furthermore, the results show that leptin pulses were independent of LH pulses induced by exogenous LHRH.

Discussion

The results of the present study, using two different interventions, suggest that leptin secretion is independent of LH secretion in ewe lambs. Studies in women during the early and midfollicular phase of the menstrual cycle have shown that LH and leptin pulses are synchronized *(13,14)*, suggesting that leptin could be a signal regulating the LHRH/LH secretions. This association was not evident in the present study because there was no synchronicity between LH and leptin pulses. It is thus not possible to affirm that LH secretion is modulated by pulses of leptin. Furthermore, the fact

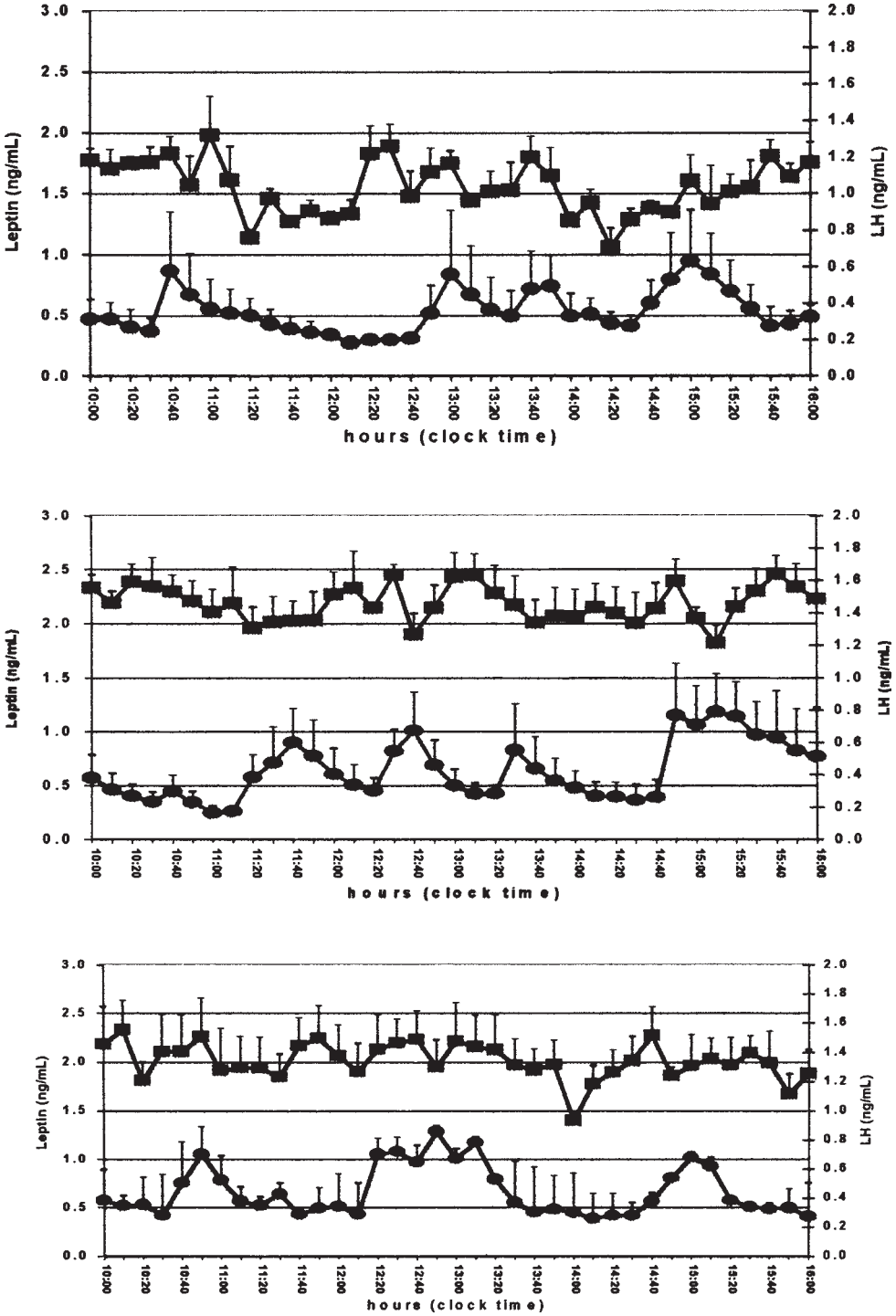

Fig. 1. Mean (±SEM) diurnal plasma leptin (■) and LH (●) concentrations in control ewe lambs at 20 (**A**), 26 (**B**), and 30 (**C**) wk of age.

that no synchronicity between LH and leptin pulses was observed suggests that leptin secretion is independent of LH secretion. There were no changes in pulsatile leptin secretion in control ewe lambs in lambs treated with a long-acting LHRH agonist that reduces the LH secretion, or in lambs treated with pulses of LHRH. These results confirm our previous observations in women with Kalmann syndrome. These women lack endogenous LHRH secretion and even though they do not have LH pulses, they exhibit a clear pattern of leptin secretion similar to that of normal cycling women *(15)*. Moreover, this pattern did not change when LHRH pulses were given at regular intervals by means of an LHRH pump to activate the gonadal axis *(15)*, clearly indicating that both hormone secretions are independent of each other.

Our results are also similar to those obtained in lactating women during the early postpartum period *(16)*, in which

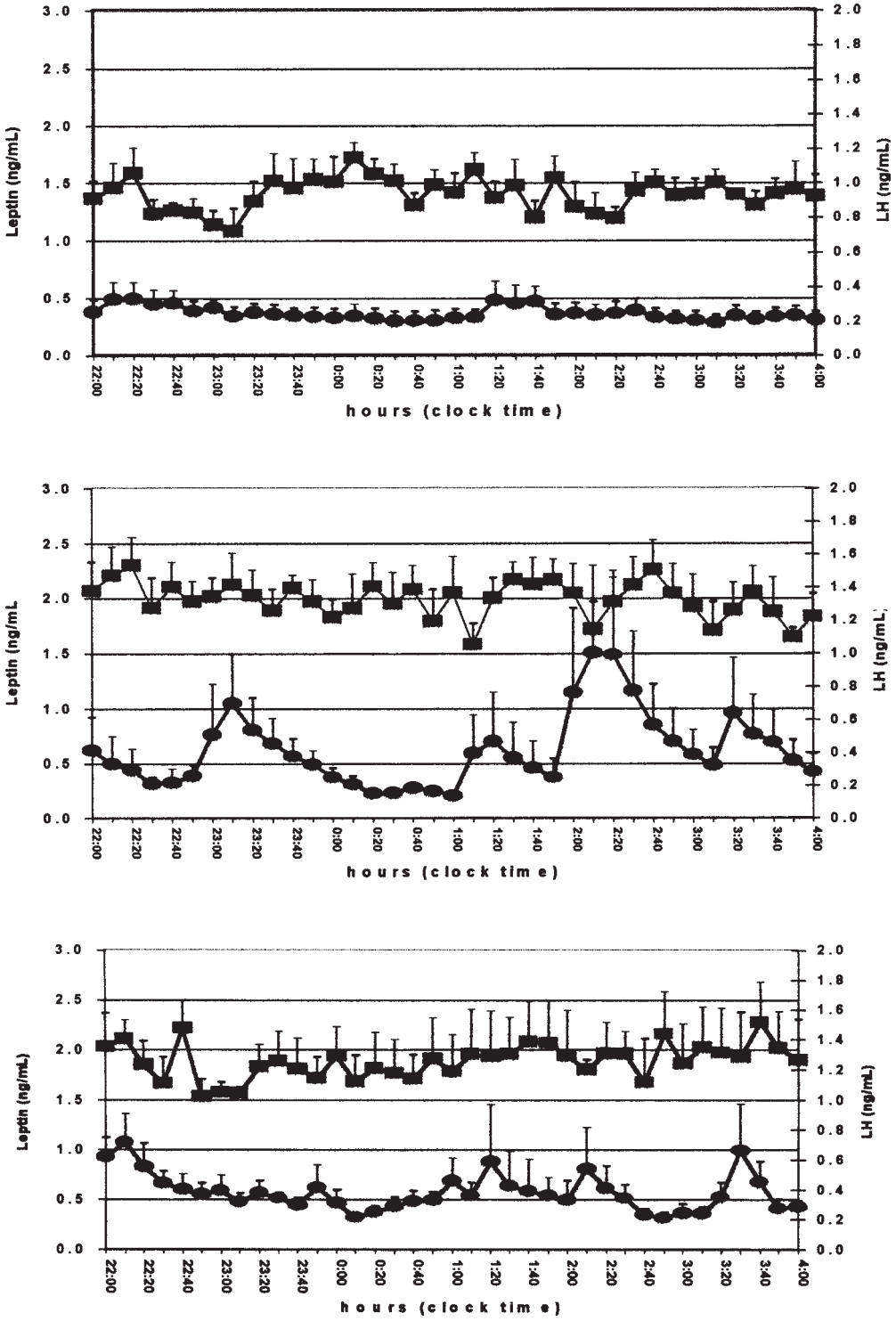

Fig. 2. Mean (±SEM) nocturnal plasma leptin (■) and LH (●) concentrations in control ewe lambs at 20 (**A**), 26 (**B**), and 30 (**C**) wk of age.

no synchronicity was observed between LH and leptin pulses. Breast-feeding women exhibited low levels of estradiol. Low levels of plasma estradiol have also been found in prepubertal female sheep *(17)*. Licinio et al. *(13)* showed that LH, leptin, and estradiol were synchronized in the midfollicular phase of the menstrual cycle in women. It is therefore possible that estradiol may be a permissive signal that could allow the coupling between the secretion of both hormones. Furthermore, it is possible that in female sheep, once the coordinating estradiol secretion becomes rhythmically established after the onset of puberty, the synchronicity between both hormones could become apparent.

Pulsatile LHRH secretion is the result of a complex neuroendocrine control that results in changes in pulse frequency and, to a lesser degree, in pulse amplitude, determining the ultradian episodic LH secretion. The long-lasting LHRH

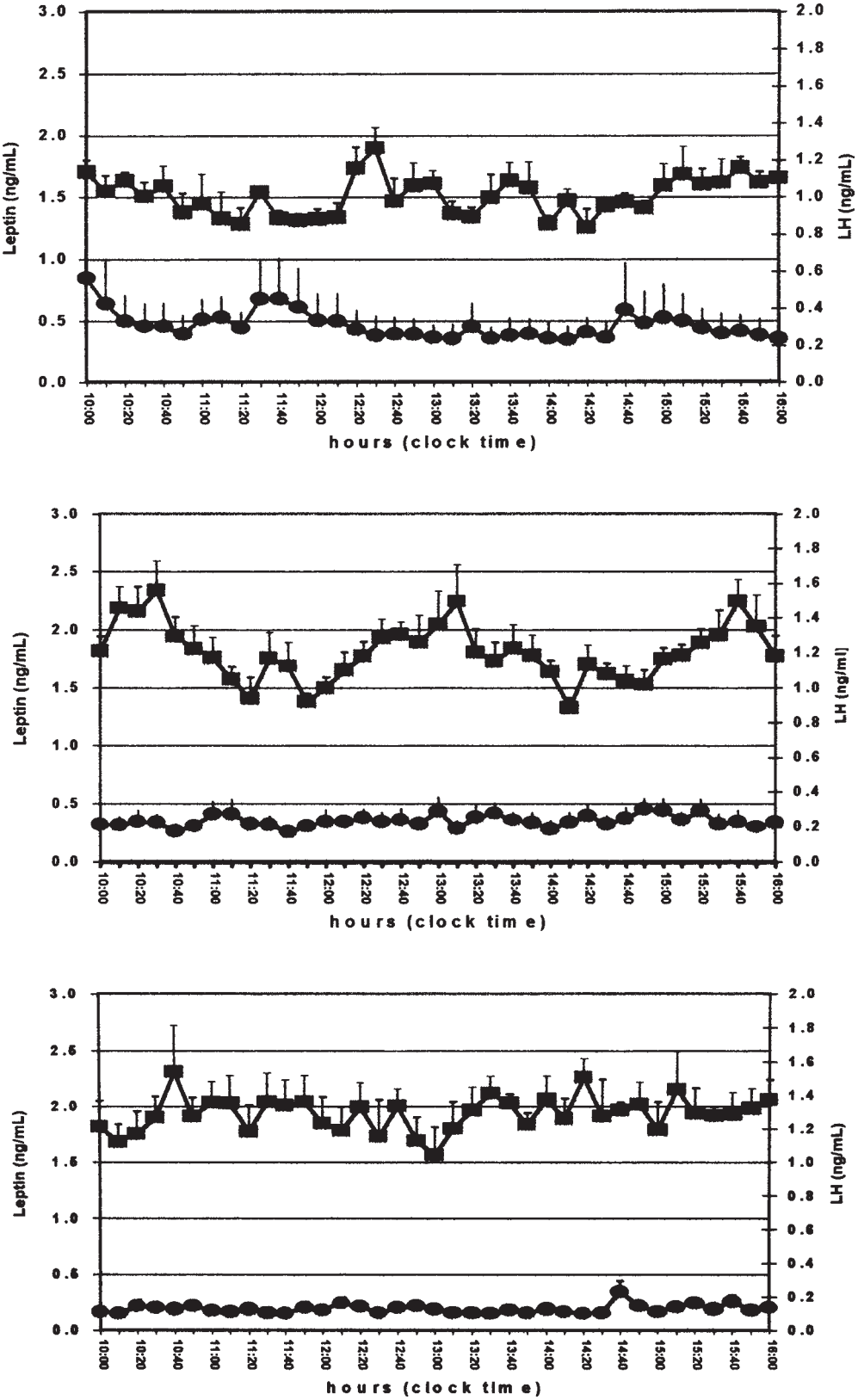

Fig. 3. Mean (±SEM) diurnal plasma leptin (■) and LH (●) concentrations in ewe lambs at 20 (**A**), 26 (**B**), and 30 (**C**) wk of age treated with a long-acting LHRH agonist.

agonist diminished LH secretion, preventing the sustained elevation in mean plasma LH concentrations observed in control lambs. At 30 wk of age, lambs treated with the LHRH agonist exhibited half the mean plasma LH of control lambs and approx 30% of the mean LH pulse amplitude and nadir concentrations. To the best of our knowledge, this is the first

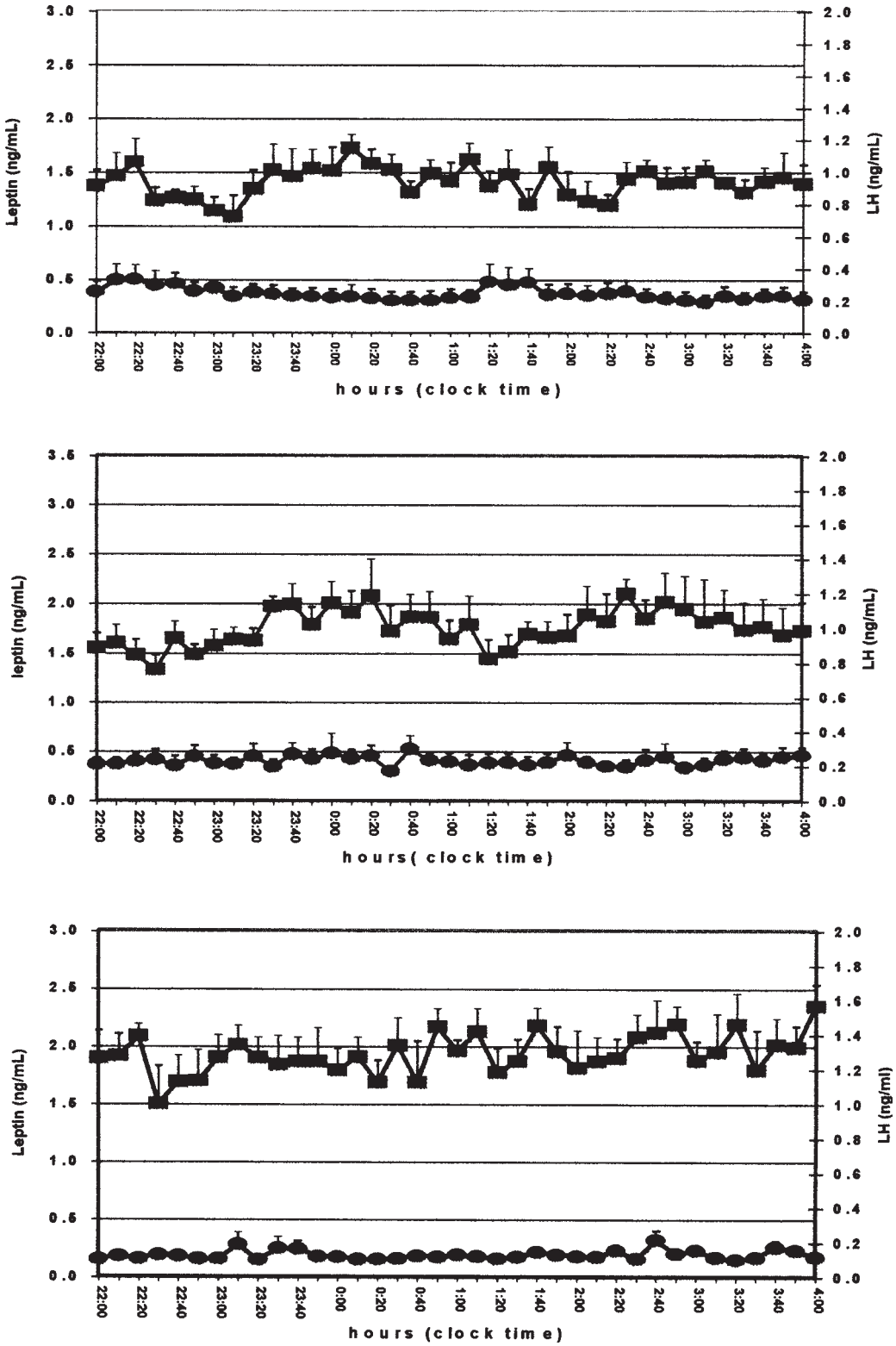

Fig. 4. Mean (±SEM) nocturnal plasma leptin (■) and LH (●) concentrations in ewe lambs at 20 (**A**), 26 (**B**), and 30 (**C**) wk of age treated with a long-acting LHRH agonist.

report about the effect of this LHRH agonist on LH secretion in ewe lambs. These results confirm earlier studies in humans *(18,19)* and rats *(12,20)* about the effect of this LHRH agonist on the release of LH and the possibility of using it as a potential tool to study the control of LH secretion during prepubertal development. The control of the ultradian,

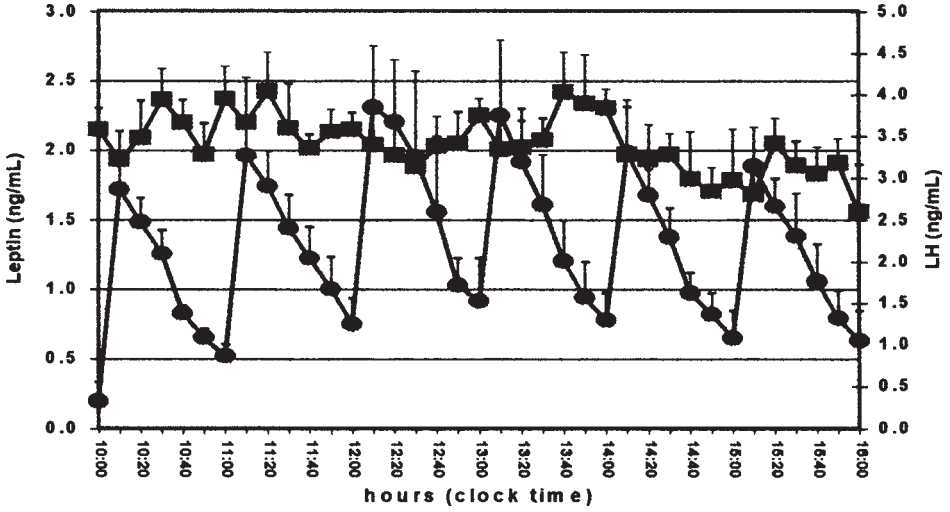

Fig. 5. Mean (±SEM) plasma leptin (■) and LH (●) concentrations in response to six LHRH pulses administered at 60-min intervals in ewe lambs at 30 wk of age. The first LHRH pulse was given at 10:00 AM.

episodic pulsatile leptin secretion is not well understood, specifically which signals drive the frequency or amplitude of the pulses. One possibility is that LH pulses might synchronize leptin pulses; however, the present study discards this possibility. A second alternative is that other stimuli, acting on the adipocyte, may regulate the frequency and amplitude of the leptin pulses. One of these driving forces may be insulin. Insulin, independent of body fat and of body mass index, is a potent stimulus on leptin secretion *(21)*. Insulin is also secreted in a pulsatile manner *(22,23)*; however, the copulsatility of both hormones has not being studied in humans or animals.

Nocturnal LH secretion is not different from diurnal secretion in normal growing lambs These results confirm previous findings of our laboratory in which it was demonstrated that ewe lambs do not show a circadian rhythm of LH during prepubertal development *(24)*. Concordant with the absence of a diurnal rhythm of LH, nocturnal parameters of pulsatile leptin secretion are similar to those of diurnal leptin secretion. This suggests that leptin does not have a discernible diurnal rhythm of secretion in prepubertal sheep, contrary to what has been proposed for prepubertal and adult humans, who exhibit increases in nocturnal plasma leptin *(8,11,25)*. Moreover, in prepubertal female rats, a nocturnal increase in plasma leptin precedes puberty by 8 d, and this increase has been postulated as an early anticipatory signal of the onset of puberty *(26)*. However, more detailed studies of the rhythm of leptin secretion in normal women have not demonstrated a change in the nocturnal secretion attributable to a circadian rhythm *(27)*. The daily rhythm of leptin has been attributed to the feeding pattern *(28,29)*, and it seems to be coupled with the circadian rhythm of cortisol and prolactin in rats and humans *(30,31)*. However, recent results in rats provide an anatomic basis for the diurnal rhythm of leptin. The circadian rhythm of leptin could depend on the activity of the suprachiasmatic nuclei *(32)*. This would mean that the rhythm of leptin secretion is directly driven from the suprachiasmatic nuclei and is not synchronized by other rhythms. The lack of concordance between our results and those found in rodents may be attributable to differences between species.

In summary, LH secretion decreased steadily, whereas leptin increased in LHRH agonist–treated lambs, with no discernible synchronicity between LH and leptin pulses. By contrast, in LHRH-stimulated lambs, plasma LH concentrations increased in response to each LHRH pulse, while no change in pulsatile leptin secretion was observed. Both observations suggest that leptin secretion is independent of LH secretion in ewe lambs. Moreover, leptin secretion seems to lack a diurnal rhythm in ewe lambs.

Materials and Methods

General Procedures

Sixteen spring-born Suffolk ewe lambs were studied. They were born at the Sheep Production Unit of the Universidad de Concepción, at the Chillán Campus; weaned at 8 wk of age; and then moved to the facilities of the Faculty of Veterinary Medicine. They were pastured and given a supplement of pelleted food twice a day. Starting from 16 wk of age, they were fed only pelleted food. At 20 wk of age, the ewe lambs were allotted to three groups: a control untreated group (n = 6), an LHRH analog–treated group (n = 5), and an LHRH-treated group (n = 5). The LHRH analog group received a dose of LHRH-Trp^6 (3.75 mg) intramuscularly at 20, 24, and 28 wk of age, in a slow releasing microcapsule preparation (Decapeptyl®, Ferring, Kiel, Germany). Four to five days before the LH and leptin study,

the ewe lambs were moved to the animal experimentation room and placed in individual cribs, with free access to pelleted food and water. Indwelling jugular vein catheters were placed under local anesthesia for collecting blood samples and for administering LHRH pulses as described elsewhere *(33)*. Blood samples were taken at regular intervals 2 to 3 d prior to the experiment, to allow the lambs to get used to the blood collection procedures and to minimize stress. The procedures were revised and approved by the local Ethics Committee on Animal Research.

Studies of Pulsatile LH and Leptin Secretion

The control group and the LHRH analog group were studied at 20, 26, and 30 wk of age. The study of episodic hormone secretion consisted of collecting blood samples from the jugular vein for 6 h at 10-min intervals. Blood collection began at 10:00 AM (diurnal sampling) and at 10:00 PM (nocturnal sampling). The nocturnal blood sampling was performed under a dim red light to avoid disturbing the nocturnal melatonin secretion. Blood samples were placed in heparinized tubes kept in ice and centrifuged at 1000*g* for 15 min. Plasma was stored frozen at –20ºC until later hormone measurements.

Administration of LHRH

The LHRH-treated group was studied at 30 wk of age only during daylight hours. This experiment consisted of iv administration of six pulses of LHRH (10 ng/kg of body wt) at 60-min intervals by means of an indwelling jugular vein catheter beginning at 10:00 AM. Blood samples were collected at 10-min intervals starting from the first LHRH pulse and ending 60 min after the last LHRH pulse, with a total time equivalent to that of the pulsatile LH and leptin secretion study (360 min). Blood samples were placed in heparinized tubes kept in ice and centrifuged at 1000*g* for 15 min at 4ºC. Plasma was separated and frozen at –20ºC until the radioimmunoassay (RIA) of LH and leptin.

Hormone Measurements

Plasma LH concentrations were determined by RIA, using ovine radioiodinated LH (LER 1374-A), ovine antiserum CSU-204, and ovine LH standard (oLH-S25) provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIADDK) in 200-µL duplicates, following procedures described elsewhere *(34)*. The intra- and interassay coefficients of variation (CVs) were 5 and 12%, respectively. The minimal detectable dose of LH, defined as 90% of the buffer control, was 0.1 ng/mL.

Plasma leptin concentrations were determined by RIA, using the Multi-species RIA kit from Linco. This kit has been used to determine plasma leptin concentrations in farm animals such as pigs *(35)*, cows *(36)*, and sheep *(37–39)*. The main difference between this multispecies assay and a recently developed ovine leptin RIA resides in sensitivity, with a coefficient of correlation of +0.87 between both, using a curvilinear adjustment *(40)*. The intra- and interassay CVs were 7 and 12%, respectively. The minimal detectable dose of leptin, defined as 90% of the buffer control, was 0.9 ng/mL.

Pulse Analysis and Statistical Evaluation

For pulse analysis, the computerized version of the cluster pulse algorithm was used *(41)*. A cluster configuration of 1x2 (one sample for the test peak and two for the test nadir), and a *t* value of 2.14/2.14 to reduce the possibilities of false positive pulse determination <5% was selected. The following mean properties of leptin and LH pulsatile concentrations were analyzed: transversal mean (ng/[mL·6 h]), pulse frequency (number of significant peaks/6 h), pulse amplitude (ng/mL), and nadir (ng/mL). For the analysis of copulsatility between LH and leptin in the control group, the ANCOPULS program *(42)* was used, as described previously *(14)*.

The transversal mean, pulse frequency, pulse amplitude, and nadir in control and LHRH agonist–treated lambs were assessed by analysis of variance for repeated measures with treatment as the main factor, and diurnal and nocturnal sampling and age as the repeated measures factor using the GB-Stat statistical program. Pairwise post-hoc comparisons were made by the Newman-Keuls test. A value of $p < 0.05$ was considered statistically significant. The results are shown as mean ± SEM.

Acknowledgments

We are grateful to Dr. G. D. Niswender, Dr. L. E. Reichert Jr., and Dr. A. F. Parlow for providing reagents for the ovine LH RIA, and to Prof. José Parilo for providing the sheep. We also thank Margarita San Martin for her help in translating the manuscript. This work was supported by Fondecyt grant 1990389.

References

1. Foster, D. L. (1988). In: *Physiology of reproduction*. Knobil, E. and Neill, J. D. (eds.). Raven: New York.
2. Vogel, G. (1996). *Science* **274,** 1466–1467.
3. Foster, D. L. and Nagatani, S. (1999). *Biol. Reprod.* **60,** 205–215.
4. Kiess, W., Blum, W. F., and Aubert, M. C. (1998). *Eur. J. Endocrinol.* **138,** 26–29.
5. Cheung, C. C., Thorton, J. E., Kuijper, J. L., Wiegle, D. S., Clifton, D. K., and Steiner, R. A. (1977). *Endocrinology* **138,** 855–858.
6. Chehab, F., Mounzih, J., Lu, R., and Lim, M. E. (1996). *Science* **275,** 88–90.
7. Lahlou, N., Landais, P., De Boissieu, D., and Bougneres, P.-F. (1997). *Diabetes* **46,** 989–993.
8. Pombo, M., Herrera-Justiniano, E., Considine, R. V., Hermida, R. C., Gálvez, M. J., Martin, T., Barriero, J., Casanueva, F. F., and Dieguez, C. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2751–2754.
9. Blum, W. F., Englaro, P., Hanisch, S., Juul, A., Hertel, N. T., Muller, J., Skakkebaek, N. E., Heiman, M. L., Birkett, M., Attanasio, A. M., Kiess, W., and Rascher, W. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2904–2910.

10. García-Mayor, R. V., Andrade, M. A., Rios, M., Lage, M., Dieguez, C., and Casanueva, F. F. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2849–2855.
11. Palmert, M. R., Radovick, S., and Boepple, P. A. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1091–1096.
12. Recabarren, S. E., Dittrich, R., Jäger, W., and Wildt, L. (1991). *Hum. Reprod.* **6,** 928–930.
13. Licinio, J., Negrao, A. B., Mantzoros, C., Kaklamani, V., Wong, M. L., Bongiorno, P. B., Mulla, A., Cearnal, L., Veldhuis, J. D., Flier, J. S., McCann, S. M., and Gold, P. W. (1998). *Proc. Natl. Acad. Sci. USA* **95,** 2541–2546.
14. Sir-Petermann, T., Piwonka, V., Pérez, F., Maliqueo, M., Recabarren, S. E., and Wildt, L. (1999). *Hum. Reprod.* **14,** 1435–1439.
15. Sir-Petermann, T., Maliqueo, M., Palomino, A., Vantman, D., Recabarren, S. E., and Wildt, L. (1999). *Hum. Reprod.* **14,** 2695–2699.
16. Sir-Petermann, T., Recabarren, S. E., Lobos, A., Maliqueo, M., and Wildt, L. (2001). *Hum. Reprod.* **16,** 244–249.
17. Ryan, K. D., Goodman, R. L., Karsch, F. J., Legan, S. J., and Foster, D. L. (1991). *Biol. Reprod.* **45,** 471–477.
18. González-Barcena, D., Pérez-Sánchez, P. L., Graef, A., Gómez, A. M., Berea, H., Comaru-Schally, A. M., and Schally, A. V. (1989). *Prostate* **14,** 291–300.
19. Lahlou, N., Carel, J. C., Chaussain, J. L., and Roger, M. (2000). *J. Pediatr. Endocrinol. Metab.* **13(Suppl. 1),** 723–737.
20. Jäger, W., Dittrich, R., Recabarren, S., Wildt, L., and Lang, N. (1995). *Tumour Biol.* **16,** 268–280.
21. Saad, M. F., Khan, A., Sharma, A., Michael, R., Riad-Gabreil, M. G., Boyadjian, R., Jinagouda, S. D., Steil, G. M., and Kamdar, V. (1998). *Diabetes* **47,** 544–549.
22. Vanhelder, W. P., Sirek, A., Sirek, O. V., Norwich, K. H., Policova, Z., and Vanhelder, T. (1980). *Diabetes* **29,** 326–328.
23. Goodner, C. J., Koerker, D. J., Stagner, J. I., and Samols, E. (1991). *Am. J. Physiol.* **260,** E422–E429.
24. Recabarren, S. E., Lobos, A., Henríquez, J., Peñeipil, C., and Parilo, J. (1998). *Agrociencia* **14,** 293–305
25. Saad, M. F., Riad-Gabriel, M. G., Khan, A., Sharma, A., Michael, R., Jinagouda, S. D., Boyadjian, R., and Steil, G. M. (1998). *J. Clin. Endocrinol. Metab.* **83,** 453–459.
26. Nagatani, S., Guthikonda, P., and Foster, D. L. (2000). *Horm. Behav.* **37,** 345–352.
27. Marsh, E. E., Taylor, A. E., Anderson, E. J., and Hall, J. E. (2000). In: *Proceedings of the Endocrine Society's 82nd Annual Meeting.* The Endocrine Society Press, Bethesda, MD. Abstract 2056, p. 498.
28. Simon, C., Gronfier, C., Schlienger, J. L., and Brandenberger, G. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1893–1899.
29. Schoeller, D. A., Cella, L. K., Sinha, M. K., and Caro, J. F. (1997). *J. Clin. Invest.* **100,** 1882–1887.
30. Nishiyama, M., Makino, S., Suemaru, S, Nanamiya, W., Asaba, K., Kaneda, T., Mimoto, T., Nishioka, T., Takao, T., and Hashimoto, K. (2000). *Horm. Res.* **54,** 69–73.
31. Mastronardi, C. A., Walczewska, A., Yu, W. H., Karanth, S., Parlow, A. F., and McCann, S. M. (2000). *Proc. Soc. Exp. Biol. Med.* **224,** 152–158.
32. Kalsbeek, A., Fliers, E., Romijn, J. A., La Fleur, S. E., Wortel, J., Bakker, O., Endert, E., and Buijs, R. M. (2001). *Endocrinology* **142,** 2677–2685.
33. Recabarren, S. E., Urrucelqui, A., Robbiano, M., Lobos, A., Orellana, P., and Parilo, J. (1995). *Arch. Med. Vet.* **27,** 99–104.
34. Recabarren, S. E., Escobar, A., Lobos, A., Recabarren, M. P., and Parilo, J. (1996). *Exp. Clin. Endocrinol.* **104,** 74–79.
35. Qian, H., Barb, C. R., Compton, M. M., Hausman, G. J., Azain, M. J., Kraeling, R. R., and Baile, C. A. (1999). *Domest. Anim. Endocrinol.* **16,** 135–143.
36. Chilliard, Y., Bocquier, F., Delaveaud, C., Guerre-Millo, M., Bonnet, M., Martin, P., Faulconnier, Y., and Ferlay, A. (1998). In: *Proceedings of the Cornell nutrition conference for feed manufacturers.* Cornell University Agricultural Experiment Station, Cornell University, Ithaca, NY, pp. 65–74.
37. Chilliard, Y., Bocquier, F., and Doreau, M. (1998). *Reprod. Nutr. Dev.* **38,** 131–152.
38. Bocquier, F., Bonnet, M., Faulconnier, Y., Guerre-Millo, M., Martin, P., and Chilliard, Y. (1998). *Reprod. Nutr. Dev.* **38,** 489–498.
39. Nagatani, S., Zeng, Y., Keisler, D. H., Foster, D. L., and Jaffe, C. A. (2000). *Endocrinology* **141,** 3965–3975.
40. Delavaud, C., Bocquier, F., Chilliard, Y., Keisler, D. H., Gertler, A., and Kann, G. (2000). *J. Endocrinol.* **165,** 519–526.
41. Veldhuis, J. D. and Johnson, M. L. (1986). *Am. J. Physiol.* **250,** E486–E493.
42. Albers, N., Bettendorf, M., Herrmann, H., Kaplan, S. L., and Grumbach, M. M. (1993). *Endocrinology* **132,** 701–709.