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SHORT NOTE [NOTA CORTA]

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LEPTIN CIRCADIAN RHYTHM EXPRESSION IN GOATS WITH DIFFERENT BODY MASS INDEX AND DURING DIFFERENT SEASONS

[EXPRESIÓN DEL RÍTMO LEPTINO CIRCADIANO EN CABRAS CON DIFERENTE ÍNDICE DE MASA CORPORAL Y DURANTE DIFERENTES ÉPOCAS]

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SUMMARY

The objective was to determine if serum leptin expresses a circadian rhythm in goats, and the effect of body mass index (BMI) and season of the year on such expression. Eight mature, ovariectomized, female goats with s.c. sylastic implants containing 17β-estradiol were maintained in a high (HBMI, n=4) or low (LBMI, n=4) BMI during the breeding (BS-december, n=8) and transition into anestrous (TS-february, n=8) seasons. Blood samples were obtained every hour throughout a 48 h period and serum leptin determined (Linco Res. Inc.). Results were analyzed by COSINOR program (fit of data to a cosine curve) obtaining the MESOR (mean leptin concentration), AMPLITUDE (half difference between highest and lowest leptin concentration) and ACROPHASE (time of highest leptin concentration). Frequency of occurrence of a circadian rhythm was analyzed by Fisher's exact test. A leptin circadian rhythm was evident ($P<0.10$) in 5/8 goats. BMI tended to influence the expression of leptin circadian rhythms ($P=0.13$); 50% (4/8) vs. 12.5% (1/8) of goats w/leptin circadian rhythm in HBMI vs. LBMI. Season did not affect ($P=0.36$) the expression of leptin circadian rhythm; 37.5% (3/8) and 25% (2/8) goats w/leptin circadian rhythm in BS and TS. Goats w/leptin circadian rhythm showed a diurnal ACROPHASE between 11:30 to 15:30 h. Frequency of leptin circadian rhythm in TS was low and similar in BMI groups (1/4 for HBMI and LBMI). There was a differential response between BMI groups during the BS with HBMI goats showing the highest frequency of leptin circadian rhythm (3/4 and 0/4 for HBMI and LBMI, $P<0.001$). The LBMI group had higher MESOR ($P<0.05$) and basal leptin concentrations (1.9 vs. 1.4 and 1.2 vs. 0.5 ng/ml in LBMI vs. HBMI). There were no differences ($P>0.05$) in AMPLITUDE for any effect or their interaction. Evidence of a serum leptin circadian rhythm in female goats, with a diurnal increase in serum leptin was found and reported for the first time. Moreover, an effect of interaction between BMI and season in the expression of such rhythm was observed. Studies involving serum leptin in goats should take into account its circadian variation; otherwise under- or over-estimations might occur.

Key words: Goats, leptin, circadian rhythm.

INTRODUCTION

Leptin is a hormone secreted mainly by adipocytes and has a role on energy metabolism and mammalian reproduction (Brann *et al.*, 2002). Circulating level of leptin may be a useful adiposity index of an individual; however, this advantage can be lost because of its variation throughout the day. Leptin circadian rhythms or diurnal variations had been described in nonruminant species (Ahrén, 2000, mice; Downs y Urbanski, 2006, monkeys; Ishioka *et al*., 2005, dogs); however, in ruminants published data are contradictory (Bertolucci *et al*., 2005; Marie *et al*., 2001; Daniel *et al*., 2002), and there is not available information for goats. In addition, leptin circulating level can be different upon the seasons of the year (Marie *et al*., 2001), but the asociation between both, season of the year and body energy status has not been reported. Thus, the objetive of this paper was to determine whether serum leptin concentrations show a circadian rhythm in female goats and evaluate the effect of the body mass index and season of the year on the expression of such rhythm.

MATERIALS AND METHODS

This study was conducted in the State of Queretaro México (20º 42’ N, 100º 01’ W). Eight mature, ovariectomized creole goats, with subcutaneous silastic implants containing 17β-estradiol were used. Goats were individually fed to mantain a body mass index (BMI), either high (HBMI $\geq$10; n=4) or low (LBMI $\leq$9.5; n=4). A food ration containing 14.1% of crude protein and 2.5 Mcal of metabolizable energy/kg of dry matter was offered daily at 08:00 h. Water was always available throughout the study.

During the breeding season (BS, December, n=8) and transition into the anestrous season (TS, February, n=8), blood samples were obtained in both BMI groups at 1 h intervals for a 48 h-period, to determine serum leptin concentration. Blood samples were mantained at 4ºC until centrifugation (3000 rpm x 15 min). Serum samples obtained were mantained at –20ºC until they were assayed for leptin concentrations by radioimmunoassay, using a multispecies kit (Linco Research, St. Charles MO, USA). The assay sensitivity was 0.34 ng/ml and the intra- and interassay coefficients of variation were 6.3 and 7.4%, respectively. Data were analized to determine the presence of serum leptin circadian rhythms, using the COSINOR program, which test the fit of data to a cosine curve, obtaining the MESOR (mean leptin concentration), AMPLITUDE (half difference between highest and lowest leptin concentration) and ACROPHASE (time of highest leptin concentration). Basal leptin concentrations were also obtained.

The incidence of serum leptin circadian rhythms between groups was analyzed by Fisher’s exact test. Mean leptin, amplitude and basal level were analized by ANOVA, using a split-plot design, including in the model the BMI as the whole-plot and season and the interaction of BMI by season as the subplot.

RESULTS

The COSINOR analysis showed evidence of the expression of a circadian rhythm (P<0.10) in serum leptin levels in five of the animals. The BMI tended (P=0.13) to affect the expression of circadian rhythms; incidence was higher in animals in the HBMI group vs LBMI (Table 1). Goats expressing a leptin circadian rhythm showed a diurnal acrophase between 11:30 and 15:30 h. There was a different response (P<0.001) between the groups of BMI during the reproductive season (Table 2); the goats in the HBMI group had a higher frecuency of circadian rhythms (75%, 3 out of 4 goats) vs LBMI group (0%, 0 out of 4 goats); however, during the transition into anestrous season, the frequency was similar for both BMI groups (25%, 1 out of 4 goats). The LBMI group had a higher mean (MESOR) and basal concentration of leptin as compared with the HBMI group, with no differences due to season or the interaction of BMI x season (Table 3). There were no effects of any factor or their interaction (P>0.05) on leptin COSINOR amplitude.

Table 1. Leptin circadian rhythms in female goats with different body mass index, during different seasons of the year.

	Body mass index[a]		Season of the year[b]	
	Low	High	Reproductive	Transition into anestrous
% goats w/circadian rhythm	12.5 (1/8)	50 (4/8)	37.5 (3/8)	25 (2/8)

[a]P= 0.13 HBMI vs LBMI [b]P= 0.36 Reproductive vs Transition

Table 2. Leptin circadian rhythms in goats with different body mass index, during different seasons of the year (P<0.001).

	HBMI-BS	LBM-BS	HBMI-TS	LBMI-TS
% goats w/circadian rhythm	75 (3/4)	0 (0/4)	25 (1/4)	25 (1/4)

HBMI= High body mass index; BS= Breeding season
LBMI= Low body mass index; TS= Transition into anestrous season

Table 3. Leptin Mesor, Amplitude and serum basal concentrations in goats with different body mass index, during different seasons of the year.

	BODY MASS INDEX		SEASON OF THE YEAR		BMI X SEASON
	Low	High	Reproductive	Transition	P
Leptin, Mesor (ng/ml)	1.94±0.16*	1.43±0.16*	1.78±0.16	1.59±0.16	0.31
Leptin, Amplitude (ng/ml)	0.20±0.08	0.32±0.08	0.21±0.08	0.30±0.08	0.26
Leptin, Basal (ng/ml)	1.24+0.21**	0.52+0.22**	1.15+0.22	0.61+0.22	0.25

*P<0.05 Low vs High **P<0.01 Low vs High

DISCUSSION

Some goats in this study showed serum leptin circadian rhythms, as has been described for other species (Bertolucci *et al*., 2005, ewes; Downs y Urbanski, 2006, monkeys). In ruminants, the contradictory results observed can be related to the feeding regime and hence the postprandial insulin secretion; *ad libitum* or frequent feeding (Daniel *et al*., 2002) could be factors which cause failures in detecting leptin circadian rhythms as has been reported in human beings (decrease in amplitude and a delay in the acrophase; Fogteloo *et al*., 2004). The higher amount of food provided only once a day to animals in HBMI group as compared to LBMI group, could also cause a higher insulin and leptin postprandial increases with enhanced leptin amplitude and expression of circadian rhythm. In the present study, leptin amplitude in the HBMI group was higher than the LBMI group; however, the difference was not significant. The higher frequency of goats expressing leptin circadian rhythms in the HBMI-BS group, may suggest a relationship between leptin levels and the reproductive status associated with different seasons (Marie *et al*., 2001). In goats which showed a leptin circadian rhythm, the leptin acrophase occurred after the feeding time in a similar way as reported in other species (Marie *et al*., 2001; Ishioka *et al*., 2005), suggesting a similar stimulation pathway to raise circulating leptin in these species. The greater mean and basal concentrations of leptin in the LBMI group, as compared with the HBMI group, could reflect a higher metabolic load induced by more food intake, and hence a greater clearance rate in the HBMI group, as has been suggested for other hormones (Vasconcelos *et al*., 2003). Although goats in the LBMI group were in a poor body condition and hence had scarce subcutaneous fat, serum leptin concentrations did not reflect their extent of body fat, but instead maybe reflected their metabolic status associated with the low food intake, as has been suggested for fasting conditions in lambs (Altmann *et al*., 2006).

CONCLUSION

This study indicates for the first time, that goats as other species, can express a serum leptin circadian rhythm but with a diurnal increase, and that this rhythm might be associated to food intake. Energy reserves (BMI) interacting with season can influence the expression of such rhythm. Studies involving serum leptin in goats, should take into account its circadian variation in order to avoid misleading results and conclusions.

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