

exon 1 might affect leptin levels in obese populations.

**Response of leptin levels to short term feeding and fasting in man, influence of circadian cycle.** M. Romon, P. Lobel, C. Le Fur, J.L. Edmé, B. Hecquet, J.C. Fruchart, J. Auwerx, J. Dallongeville (Inserm U 325, Institut Pasteur Lille, Service de nutrition CHU, 59045 Lille cedex, France).

**Introduction:** It has been suggested that leptin levels vary according to a nycthemeral cycle. We hypothesize that this variation could be due to a feeding/fasting cycle. We investigated whether fasting and acute feeding induced changes in circulating leptin levels in humans and whether these changes varied according to a nycthemeral cycle. meal.

**Methods:** Thirteen healthy subjects (BMI  $22.3 \pm 1.7$  kg/m<sup>2</sup>) were given either a mixed meal ( $4.6 \pm 0.2$  MJ) or remained fasting at night or during the day. Six hours before the beginning of each session, either fed or fasted, they were given the same light meal ( $2.3 \pm 0.8$  MJ). Blood samples were drawn at baseline and hourly for 8 h. Leptin response was calculated as the sum of the differences between baseline levels and hourly responses. Comparisons were made by a two-way analyses of variance with repeated measures, followed by the Dunnett test.

**Results:** Serum leptin levels increased after the test-meal ( $+41.8 \pm 38\%$ ;  $P < 0.03$ ) and decreased in the fasting state ( $-29 \pm 15\%$ ;  $P < 0.05$ ). There were no statistically significant differences in the leptin responses to the test meal between the night and day sessions ( $40 \pm 22$  versus  $41.8 \pm 38\%$ ) or to fast ( $-22 \pm 11$  versus  $-29 \pm 15\%$ ).

**In conclusion,** in non-obese subjects, serum leptin levels increased following food intake. This effect was not influenced by nycthemeral cycle.

**Photoperiod and nutritional status modulate the expression of the gene encoding leptin in ovine perirenal adipose tissue.** M. Bonnet<sup>a</sup>, Y. Faulconnier<sup>a</sup>, F. Bocquier<sup>a</sup>, P. Martin<sup>b</sup>, Y. Chilliard<sup>a</sup> (<sup>a</sup>Laboratoire sous-nutrition des ruminants, Inra, 63122 Saint-Genès-Champanelle; <sup>b</sup>Laboratoire génétique biochimique et cytogénétique, Inra, 78350 Jouy-en-Josas, France).

Leptin, a protein secreted by adipocytes, plays a major role in the regulation of food intake as well as adiposity in rodents and, in addition, modulates their reproductive cycle. Among livestock animals, sheep are one of the more sensitive to photoperiod, which effects both their reproductive cycles as well as their food intake. It was therefore decided to study the respective effects of photoperiod and nutritional status on the expression of the gene encoding leptin in ovine adipose tissue. The experiment was conducted on four groups of five adult, dry, ovariectomized ewes, that were subjected either to a short (S; 8 h/d light) or to a long (L; 16 h/d light) photoperiod, for 3 weeks. Then, all the ewes were underfed (U) to 25 % of their maintenance energy requirements (MER) for 7 days, and half of them were slaughtered (groups S-U and L-U), while the remaining were refed (R) for 14 days at 200 % of MER (groups S-R and L-R). Leptin mRNA level was estimated semi-quantitatively by RT-PCR, and simultaneously the level of cyclophilin transcript was determined as an internal control, because the relevant gene appeared to be invariably expressed. After 28 or 40 cycles of amplification with primers specific to cyclophilin or leptin, respectively, the PCR products were fractionated and visualized on an agarose gel (3 %) stained by ethidium bromide. The leptin signal was increased both by photoperiod ( $+65\%$ ;  $P < 0.03$ ) and refeeding ( $+44\%$ ;  $P < 0.10$ ) and the effects of these two factors were additive (0.89, 1.86, 1.64 and 2.33 in

groups S-U, L-U, S-R and L-R, respectively), whereas the cyclophilin signal remained unchanged. Our results suggest that both photoperiod (at a given food intake) and nutritional status regulate the expression of the gene encoding leptin in ovine adipose tissue, at least in part through pretranslational mechanisms.

**Nutritional regulation of lipoprotein lipase activity and its messenger RNAs in ewe adipose tissue and heart.** M. Bonnet<sup>a</sup>, J.F. Hocquette<sup>b</sup>, Y. Faulconnier<sup>a</sup>, J. Fléchet<sup>a</sup>, F. Bocquier<sup>a</sup>, Y. Chilliard<sup>a</sup> (<sup>a</sup>Laboratoire sous-nutrition des ruminants; <sup>b</sup>Laboratoire croissance et métabolismes des herbivores, Inra, 63122 Saint-Genès-Champanelle, France).

The regulation of circulating triacylglycerol (TG) uptake by adipose tissue (AT) or by muscle is a part of an animal's adaptation to fluctuations in their nutritional or physiological status. It was thus interesting to obtain a better knowledge of the factors involved in TG partitioning between these two tissues. This is regulated, at least partly, by the lipoprotein lipase (LPL) activity. LPL activity and the levels of its mRNAs were assayed in perirenal AT and cardiac muscle (CM) of ten adult, dry and non-pregnant ewes. All animals were restricted to 25 % of their maintenance energy requirement (MER) for 7 days, then half of them ( $n = 5$ ) were refed to 200 % MER for 14 days before slaughter. Refeeding increased the LPL activity (expressed per gram of tissue) in both AT (+357 %;  $P < 0.001$ ) and CM (+45 %;  $P < 0.05$ ). Similar trends were observed when the LPL activity was expressed either by whole tissue or by cell. Thus, contrary to previous observations in the rat, refeeding regulated ovine CM LPL activity in the same way as AT LPL activity, although with a smaller effect than in CM. Moreover, northern-

blot analyses using an ovine LPL cDNA revealed an increase in LPL mRNA levels after refeeding, both in AT (lack of signals for all the restricted ewes versus strong signals for all the refed ewes) and CM (+140 %;  $P < 0.02$ ). In conclusion, nutritional regulation of LPL gene expression seems to be carried out in the same way in ewe perirenal AT and CM, and, at least partly, by pretranslational mechanisms. The different regulation of CM LPL between ewes and rats probably arises from the peculiarities of nutrient digestion and absorption, and liver lipogenesis, in ruminant species.

**Effects of the infusion of  $\beta$ -,  $\beta$ 2- or  $\beta$ 3-adrenergic agonists or epinephrine on in situ lipolysis in ewe subcutaneous adipose tissue.** A. Ferlay<sup>a</sup>, C. Charret<sup>a</sup>, J. Galitzky<sup>b</sup>, M. Berlan<sup>b</sup>, Y. Chilliard<sup>a</sup> (<sup>a</sup>Laboratoire sous-nutrition des ruminants, Inra, 63122 Saint-Genès-Champanelle; <sup>b</sup>Inserm U317, Faculté de médecine, Toulouse, France).

An in vivo study of lipolysis is puzzling because changes in plasma glycerol concentrations depend both on the lipolytic activity of adipose tissues and on its utilization by non-adipose tissues. The microdialysis technique makes it possible to study in situ the regulation of lipolysis, which has been rarely investigated in ruminants. Twelve Lacaune ewes (body weight 78 kg and body condition score 3.9 on a 0–5 scale) were underfed at 60 % of their energy requirement for maintenance for 4 days, before the insertion of four probes (Carnegie, 0.5 × 20 mm) in the rump subcutaneous adipose tissue of each animal. The probes were perfused at 5  $\mu$ L/min for 120 min with 3  $\beta$ -adrenergic ( $\beta$ -A) agonists: isoproterenol (ISO, non-selective  $\beta$ -A), terbutaline (TER,  $\beta$ 2-A), CL316243 ( $\beta$ 3-A, Wyeth-Ayerst, USA) or epinephrine (EPI) at  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$