Influence of food intake timing on daily variations of leptin and other metabolic variables in nzw rabbits

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Introduction The 16kDa peptide hormone leptin is an adipose tissue-derived regulator of food intake and energy homeostasis, and a signal of the status of body energy stores to the brain. Plasma levels of leptin reflect body fat mass in humans, rodents and ruminants (Houseknecht *et al.*, 1998; Delavaud *et al.*, 2000). The aim of this study was to investigate circadian rhythms of plasma leptin and other metabolic variables in rabbits, to assess the influence of the timing of food intake and to investigate the relationship between leptin and lipid metabolites.

Materials and methods Forty-eight male New Zealand White rabbits, 2.5 kg (SEM ± 13 g), housed individually with light from 08.00 to 20.00 h, and fed a commercial diet, were divided into two age and weight matched groups. One had access to feed 20.00-08.00 h (group N) and the other 08.00-20.00 h (group D). Individual feed intake was monitored every 4 h. On the 16th day, blood samples from different groups of four animals were taken every 4 h over the ensuing 24 h, after recording rectal temperature. Leptin (Linco Research Inc., St Charles, MO, USA), corticosterone (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) and somatostatin (Hilsted L. et Holst J.J. 1982. *Reg. Peptides* **4**: 13-31) were determined by RIA on plasma (Na-EDTA as anticoagulant and aprotinin as protease inhibiter). Plasma metabolites were determined by enzymatic-colorimetric methods (Boehringer Mannheim GmbH, Mannheim, D). The data were analysed by periodic regression (Bingham C. et al. 1982. *Chronobiol*. **9**: 397-439) to reveal circadian periodicity and to estimate parameters for best-fit cosine curves for the data of each group. The F test was used to test the zero amplitude hypothesis and the validity of the cosine model at the probability level of 0.05. The cosine function was $y = y + A\cos(\omega t + \varphi)$, in which y is the mean of the physiological variable, A the amplitude, ω the angular velocity (here 360°/24h), t is the time (independent variable; hours after the reference time 00.00 h) and φ is the acrophase (time, in degrees from reference point, at which the cosine function attains its maximum).

Results Body weights and quantities of food ingested did not differ significantly between the two groups over the experimental period. The other variables showed significant 24 h sinusoidal variations in both groups, in all cases similar to those illustrated in Fig. 1 for leptin. Since the variations in group N were shifted by about 12 hours relative to those in group D, we pooled the data from the two groups, using as time reference (00.00 h) the onset of the feeding period (Table 1). On both feeding schedules, leptin, somatostatin and urea levels and body temperature peaked about 10 hours after the start of feeding; triglyceride and phospholipid levels peaked soon after feeding stopped; NEFA and corticosterone levels peaked at the end of the fasting period. Leptin levels correlate inversely with NEFA and corticosterone and directly with triglyceride, phospholipid and somatostatin (Table 2).

Table 1 Parameters of circadian rhythms of variables analysed by periodic regression in rabbits fed 12 hours a day. Data are synchronized to onset of feeding period

VARIABLE	MEAN	SEM	A	% RHYTHM	ACROPHASE (h)	P
Body temperature °C	39.71	0.05	0.27	23.3	8:19	<0.001
Leptin µg/l	3.03	0.08	0.46	30.8	11:08	<0.001
Somatostatin pmol/l	15.40	0.16	5.24	18.3	9:55	0.01
Corticosterone µg/l	25.36	0.82	7.34	47.1	22:47	<0.001
NEFA µmol/l	161.2	8.88	44.3	31.7	21:16	<0.001
Triglyceride mmol/l	1.05	0.06	0.19	13.8	13:45	0.03
Phospholipid mmol/l	0.93	0.03	0.10	11.5	13:58	0.06
Urea mmol/l	5.75	0.15	1.34	46.2	7:58	<0.001
Glucose mmol/l	10.9	0.30	1.04	21.1	16:46	<0.01

P= Likelihood of finding an F value the same as that observed if there were no variations in circadian patterns

Table 2 Correlations of leptin with:

	r	P
Body temperature	0.499	<0.05
Somatostatin	0.878	<0.01
Corticosterone	-0.922	<0.01
NEFA	-0.715	<0.05
Triglyceride	0.616	<0.05
Phospholipid	0.639	<0.05
Glucose	0.154	ns
Urea	0.625	<0.05

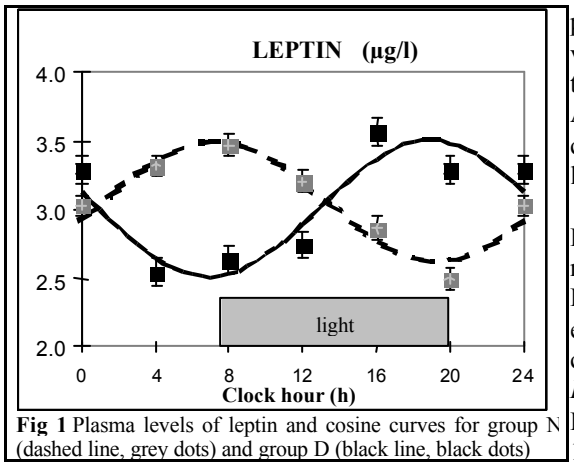

Fig 1 Plasma levels of leptin and cosine curves for group N (dashed line, grey dots) and group D (black line, black dots)

Conclusions The data of this study show clearly that the time of administration of food is a potent synchronizer of the circadian patterns of leptin and the other parameters investigated. The diurnal variations in leptin therefore depend on the "nutritional status" of the animal, rather than light cues, as Schoeller *et al.* (1997) and Ahima *et al.* (1996) also inferred in humans and mice. Leptin correlations with the variables investigated indicate that in rabbits leptin is an index of the lipid metabolism.

References Ahima, R.S., et al. 1996. Role of leptin in the neuroendocrine response to fasting. *Nature* **382**: 250-252. Delavaud, C., et al. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *Journal of Endocrinology* **165**: 519-526. Houseknecht, K.L., et al. 1998. Biology of leptin: a review. *Journal of Animal Science* **76**: 1405-1420. Schoeller, D.A., et al. 1997. Entrainment of the diurnal rhythm of plasma leptin to meal timing. *Journal of Clinical Investigation.* **100**: 1882-1887.