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Diurnal variations of serum leptin in dogs: effects of fasting and re-feeding

K. Ishioka [a,*], H. Hatai [a], K. Komabayashi [a], M.M. Soliman [a], H. Shibata [b], T. Honjoh [b], K. Kimura [a], M. Saito [a]

[a] *Laboratory of Biochemistry, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*
[b] *Morinaga Institute of Biological Science, 2-1-1 Shimosueyoshi 2, Tsurumi, Yokohama 230-8504, Japan*

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Abstract

Leptin is a protein synthesized and secreted primarily by adipocytes, and plays a key role in the regulation of energy balance. We have reported that serum leptin is elevated in obese dogs. In the present study, we examined diurnal variations of serum leptin in the dog, with special references to feeding and fasting cycles. Four male beagles were accustomed to feed once a day at 10:00 h, and blood samples were taken every 3 h for 24–36 h. Serum leptin concentration showed clear diurnal variations, being lowest before food intake (2.3 ± 0.5 ng/mL) at 09:00 h, and highest (10.5 ± 2.4 ng/mL) at 18:00 h. Such diurnal variations disappeared when the dogs were fasted. Serum insulin also showed diurnal variation with higher levels at 12:00–15:00 h. When insulin or glucose was injected in the fasted dogs to mimic the post-prandial insulin rise, serum leptin concentration was significantly increased in 4–8 h, but in both cases to a lesser extents than those after food intake. The results indicate that serum leptin concentrations change diurnally in association with feeding–fasting cycles in the dog, partially due to changes in insulin secretion.

Keywords: Dogs; Food intake; Glucose; Insulin; Leptin

1. Introduction

Leptin, the *ob* gene product, is a 16 kDa protein synthesized and secreted primarily by adipocytes (Friedman and Halaas, 1998). In humans and rodents, serum leptin concentration is known to positively correlate with body fat content, being higher in obesity (Maffei et al., 1995; Mizuno et al., 1996). In addition, serum leptin concentration shows diurnal changes in association with feeding–fasting cycles (Boden et al., 1996; Schoeller et al., 1997; Ahima et al., 1998). These variations of serum leptin may be due to the net effects of various neuroendocrine and nutritional factors such as glucose, insulin, glucocorticoids and catecholamines, which regulate synthesis of leptin in adipocytes (Wabitsch et al., 1996).

In companion animals, as in human medicine, obesity has become the most common nutritional disorder (Edney and Smith, 1986; Markwell et al., 1990). However, there have been a limited number of reports on leptin in companion animals such as the dog and cat. Recently, we have cloned canine (Iwase et al., 2000a) and feline (Sasaki et al., 2001) leptin cDNAs, produced recombinant leptin in *Escherichia coli*, and established enzyme-linked immunosorbent assay (ELISA) methods for serum leptin in the dog (Iwase et al., 2000b) and cat (Shibata et al., 2003). Using our ELISA methods, we have confirmed a highly positive relationship between serum leptin concentration and body fat content in dogs (Ishioka et al., 2002a; Sagawa et al., 2002) and cats (Shibata et al., 2003), suggesting that plasma leptin is a quantitative diagnostic marker of adiposity and obesity. In the present study, we examined diurnal variations of

* Corresponding author. Tel.: +81-11-706-5204; fax: +81-11-757-0703.
E-mail address: katsumi@vetmed.hokudai.ac.jp (K. Ishioka).

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serum leptin concentration in four healthy dogs, particularly focusing on their temporal relationship to feeding and fasting cycles. The possible roles of insulin and glucose in the diurnal variations were also investigated. This is the first report of post-prandial changes in serum leptin in dogs.

2. Materials and methods

2.1. Dogs

Four male beagles (two years old, weighing 11.6–12.4 kg) were used. They were housed in a temperature (23 ± 2 °C) and light (lights on at 06:00–18:00 h) controlled room and fed on a standard dry food (Field, Petline) once daily at 10:00 h. All dogs ate the food within 5 min after it was offered. Water was available ad libitum. Prior to each experiment, general health examinations including complete blood count (RBC, WBC, and PLT) and biochemical profiles (BUN, Creatinine, glucose, TP, AST, ALP) were performed to confirm there were no apparent abnormalities. Animal care and procedures were in accordance with the guidelines of the Animals Care and Use Committee of Hokkaido University.

2.2. Experiment 1: diurnal variations of serum leptin and food intake

Four beagles were divided into two groups (Groups A and B, two dogs in each). In the first trial, Group A was fed daily at 10:00 h as in the previous days, while Group B was fasted on the first day but fed at 10:00 h on the second day. Blood samples were taken from the cephalic vein at 09:00, 12:00, 15:00, 18:00, 21:00 h of Day 1, and 01:00, 05:00 and 09:00 h of Day 2. For Group B, blood was collected further at 12:00, 15:00, 18:00 and 21:00 h of Day 2. Serum was separated by centrifugation and stored at −20 °C. After a recovery period of two weeks, a second trial was performed by exchanging the treatments: i.e. Group B was fed as in the previous days, whereas Group A was fasted on the first day and fed on the second day, and blood samples were taken as in the first trial. Data of the two trials were analyzed as a cross-over study using the same four dogs.

2.3. Experiment 2: effects of insulin and glucose on serum leptin

One month after Experiment 1, the same four beagles were injected subcutaneously (s.c.) with isophane insulin (0.1 IU/kg, NPH, Novolin N40, Novo Nordisk) at 09:00 h. Blood samples were collected every 1 h for 12 h, during which time no food was given. Serum was separated by centrifugation and stored at −20 °C.

One month later, the same four beagles were given glucose (1 g/kg) by continuous injection of a 20% glucose solution for 1 min through a catheter in the cephalic vein at 09:00 h. This was repeated for five times at 30 min intervals: i.e. dogs were given 5 g/kg glucose during the 2 h period. Blood samples were collected every 1 h for 9 h, during which time no food was given. Serum was separated by centrifugation and stored at −20 °C.

2.4. Assays of serum leptin and other parameters

Serum leptin concentrations were measured by the previously reported method of sandwich ELISA using an anti-canine leptin antibody (Iwase et al., 2000b). Serum glucose and non-esterified fatty acids (NEFA) were assayed using respective kits (Glucose B test, and NEFA C test, Wako). Serum insulin and cortisol concentrations were measured by ELISA (Insulin ELISA kit, Morinaga), and radioimmunoassay (Amerlex Cortisol RIA kit, Amersham Corp.), respectively.

2.5. Data analysis

Data are presented as means ± SE for four dogs, and analyzed by one-way ANOVA, with a post hoc testing by Neuman–Keuls test.

3. Results

3.1. Experiment 1: diurnal variations of serum leptin and their relation to food intake

Fig. 1 shows the mean concentrations of serum leptin for four dogs in relation to circadian clock times. Serum leptin showed clear diurnal variations: i.e. it was 2.3 ± 0.5 ng/mL at 09:00 h, began to increase thereafter, reached a maximum level (10.5 ± 2.4 ng/mL) at 18:00 h, before returning to basal levels at 05:00 and 09:00 h on the following day. Such diurnal variations were not seen when the dogs were fasted: i.e. the serum leptin concentration decreased gradually to those lower than 2.0 ng/mL. However, when dogs were re-fed at 10:00 h on the next day, the leptin concentrations increased at 15:00–21:00 h in the same way as in the regularly fed dogs. Thus, serum leptin showed diurnal variations associated with feeding–fasting cycles, being increased 5–8 h after food intake.

Post-prandial changes in serum glucose, NEFA, insulin and cortisol were also examined (Fig. 2). Serum glucose and cortisol showed no consistent variations in both fed and fasted dogs. The serum NEFA concentrations increased during fasting, but decreased after food intake. In contrast, serum insulin concentrations were low during fasting, but rose rapidly after food in-