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## Plasma leptin responses to lipopolysaccharide and tumor necrosis factor $\alpha$ in cows

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### Abstract

Peripheral administration of bacterial lipopolysaccharide (LPS) and various inflammatory cytokines to rodents is known to raise plasma levels of leptin, a potent satiety factor secreted from adipocytes, implying a role of leptin in endotoxin-induced anorexia. We previously reported no effect of LPS on serum leptin levels in sheep, despite marked anorexia and fever. Our results suggest that leptin might not be involved in the endotoxin-induced anorexia in ruminants. To test this idea, in the present study, plasma leptin levels were measured during acute experimental endotoxemia in Holstein cows. Intravenous injection of LPS induced anorexia accompanied with increases in plasma levels of cortisol and insulin, all of which are known to stimulate leptin secretion in rodent and human, while it did not affect plasma leptin levels at all in cows. Similar results were also obtained after injection of recombinant bovine tumor necrosis factor  $\alpha$ . These results indicate that plasma leptin levels in cows during acute endotoxemia are differentially regulated from those in rodents, and that leptin might not be involved in the endotoxin-induced anorexia in ruminants.

Key words : anorexia ; cow ; leptin. LPS. TNF $\alpha$

### Introduction

Leptin, the product of the *ob* gene, is a cy-

tokine secreted by adipocytes. It is a potent satiety factor and also involved in the metabolic regulation of energy substrates such as

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glucose and fatty acids<sup>11,13</sup>). Circulating leptin levels are well correlated with nutritional status and body fatness, and leptin synthesis in adipocytes is influenced by various neuroendocrine factors including insulin, glucocorticoids and catecholamines. Although the regulation of food intake and nutrient metabolism in ruminants is known to be considerably different from those in non-ruminant species, quite similar properties of leptin have been reported in ruminants and non-ruminant species (for review, see ref. 4). For example, recombinant ovine leptin results in reduced food intake in sheep and goat when given intracerebroventricularly<sup>5</sup>. Plasma leptin levels are higher in well-nutritioned sheep and cows with higher body fatness<sup>3,8</sup>. Moreover, leptin expression in adipose tissue of sheep and cow is up-regulated by insulin and glucocorticoids<sup>9</sup>. All these results observed in ruminant species are essentially the same as those in mice and humans.

It has been reported in rodents that peripheral administration of bacterial lipopolysaccharide (LPS) and various inflammatory cytokines causes an increase of plasma leptin levels, implying a role of leptin in endotoxin-induced anorexia<sup>10,12,18</sup>. However, we found in sheep that intravenous injection of LPS produced no change in serum leptin levels despite marked anorexia and fever<sup>19</sup>. Our results indicate that serum leptin levels in sheep during acute endotoxemia are differentially regulated from those in rodents, and suggest that leptin might not be involved in the endotoxin-induced anorexia in ruminants. To test this hypothesis, in the present study, we examined plasma leptin responses to LPS in another ruminant species, the bovine. The effects of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) were also examined.

### Materials and Methods

Experimental procedure was in accordance with the guideline of the animal use regulation of Hokkaido University and also with those of the Tohoku National Agricultural Experimental Station.

In Experiment 1, six Holstein cows weighing 450-550kg were used to examine the effects of fasting on the plasma leptin concentration. They were housed in individual stalls in an animal facility of Hokkaido University with free access to water and a trace mineral block, and given a mixture of forage (orchard-grass hay, alfalfa hay cube, corn silage) and concentrate. Before and 24 hr after fasting, about 5ml of blood was collected from the jugular vein. Plasma was separated by centrifugation and stored at -20°C until assay.

In Experiment 2, the same six cows as in Experiment 1 were used to examine the leptin responses to LPS injection. On the day of experiment, they were divided into two groups (Group A and B, three in each), and Group A was injected intravenously with 2ml of a LPS (*Escherichia coli* 0555 : B 5, Difco, Detroit, MI, USA) solution dissolved in saline at a dose of 500 ng/kg, while Group B with saline as a control. Before and 0.5-8 hr after the injection, blood was collected from the jugular vein and plasma was stored at -20°C until assay. After one week, the treatments were exchanged: that is, Group A was injected with saline and Group B with LPS. The data obtained from the two series of experiment were pooled and treated as those from 6 cows in each treatment.

In Experiment 3, six Holstein cows, weighing about 350 kg, housed in the Tohoku National Agricultural Experimental Station were used to examine the leptin response to TNF $\alpha$  injection. The experimental procedure was same as described previously<sup>15</sup>. Briefly, the cows were injected intravenously with either highly purified recombinant bovine

TNF $\alpha$  (5  $\mu$ g/kg), which was produced by a *Bacillus brevis* host-vector system<sup>15)</sup>, or saline as a control. Before and 1-12 hr after the injection, blood was collected and plasma was stored at -20°C until assay.

Plasma leptin was assayed using multi-species leptin RIA kit (LINCO Research Inc., St. Charles, MO, USA). Although human recombinant leptin was used as a standard, the accuracy of the assay in bovine plasma was confirmed using recombinant bovine leptin as a standard (Kawakita, Y. and Abe, H., unpublished observation). Plasma cortisol, insulin and glucose concentrations were measured using a cortisol RIA kit (Amersham Corp, Arlington Heights, IL, USA), a RIA kit for human insulin (Eiken Chemical Co., Tokyo), and an automatic analyzer (COBAS MIRA, F. Hoffmann-La Roche, Basel, Switzerland), respectively. Data are represented as means  $\pm$  SEM and analyzed by Dunnett's *t*-test for multiple comparisons.

## Results and Discussion

It has been well established that circulating leptin level and leptin mRNA levels in adipose tissues are decreased after fasting in ruminants as well as in non-ruminants species such as rodents and human.<sup>1,4,14,16,20)</sup>

To confirm these previous reports and also the validity of the present leptin assay method, in Experiment 1, six Holstein cows were fasted for 24 hr and plasma leptin levels were measured using a multi-species leptin RIA kit and human recombinant leptin as a standard. As summarized in Table 1, 24-hr fasting resulted in a decrease in plasma insulin and an increase in plasma free fatty acids. In parallel with these metabolic changes, plasma leptin level decreased significantly by about 30%. Quite similar results were also reported by Amstalden et al.<sup>1)</sup>, who used a RIA highly specific to ovine leptin. Thus, plasma leptin levels

are under short-term influences of feeding and fasting in cows, similarly in non-ruminants. The results also suggest the validity of the present assay using multi-species leptin RIA kit, at least for studies of plasma leptin responses to nutritional and other challenges.

Peripheral administration of LPS and various inflammatory cytokines into rodents was shown to increase plasma leptin levels, implying a role of leptin in endotoxin-induced anorexia<sup>10,12,18)</sup>. However, we found in sheep that LPS administration produced no changes in serum leptin levels despite marked anorexia and fever, suggesting that, unlike in rodents, leptin might not be involved in the endotoxin-induced anorexia in ruminants.<sup>19)</sup> To confirm this, in Experiment 2, the effects of LPS injection on plasma leptin levels were examined using the same cows as in Experiment 1. Cows injected with LPS were tachypenic and recumbent with intermittent cough within an hour after LPS injection. They also developed anorexia and did not have any food during the experimental period. Thus it was confirmed that the LPS used in the present study effectively induced typical acute inflammatory symptoms, including anorexia, in cows. However, as shown in Fig. 1, the leptin level in the plasma from these cows was not changed for 8hr, showing almost the same levels as

Table 1. Effect of starvation on plasma levels of glucose, insulin, free fatty acids (FFA) and leptin in cows. Blood samples were taken from Holstein cows before and after 24 hr fasting. Values are means  $\pm$  SEM for 6 cows. \**p* < vs. before starvation

	Before starvation	24hr starvation
Glucose (mg/dl)	70.6 $\pm$ 2.9	61 $\pm$ 2.4
Insulin ( $\mu$ U/ml)	19.3 $\pm$ 3.9	12.5 $\pm$ 2.3*
FFA ( $\mu$ Eq/l)	315.1 $\pm$ 157.6	690.6 $\pm$ 59.7*
Leptin (ng/ml)	8.9 $\pm$ 0.5	6.3 $\pm$ 0.5*

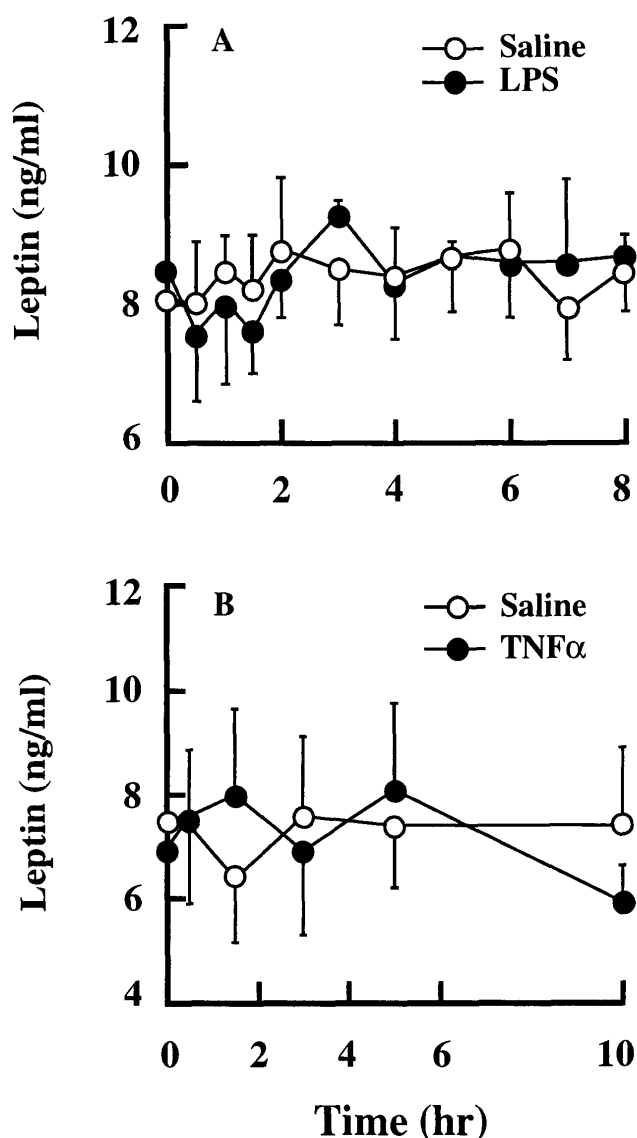


Fig. 1. Changes in plasma leptin levels after LPS or  $\text{TNF}\alpha$  injection in cows. A: Either LPS (500 ng/kg) or saline was injected intravenously, and blood samples were obtained for 8 hr. B: Recombinant bovine  $\text{TNF}\alpha$  (5  $\mu\text{g}/\text{kg}$ ) or saline was injected intravenously, and blood samples were obtained for 12 hr. Values are means  $\pm$  SEM for 6 cows.

those in saline-injected controls. These results seem to contrast with those reported in rodents, where plasma leptin levels and leptin mRNA expression in adipose tissue are increased following LPS treatment<sup>10,12,18</sup>.

In rodents, it has been reported that

chronic treatment with some inflammatory cytokines such as interleukin-1 (IL-1) and  $\text{TNF}\alpha$  result in anorexia and weight loss<sup>2</sup>. Moreover, these cytokines as well as LPS raise plasma leptin levels several hours after administration in mice<sup>18</sup>. Based on these rodents data, we examined the acute effects of  $\text{TNF}\alpha$  on plasma leptin levels in cows (Experiment 3). As shown in Fig. 1, intravenous administration of recombinant bovine  $\text{TNF}\alpha$  did not produce any significant change in the plasma level of leptin at least in 12 hr.

The plasma level of leptin is primarily dependent on its synthesis and secretion in adipocytes, which are under the influence of various neuroendocrine factors. Particularly, insulin and glucocorticoids are known as potent stimulators and catecholamines as suppressors<sup>11,13</sup>. To examine possible involvement of these factors in the regulation of plasma leptin in cows, we measured their plasma levels after injection of LPS or  $\text{TNF}\alpha$ . Insulin itself and glucose metabolism activated by insulin are suspected to play critical roles in the post-prandial rise of plasma leptin level in rodents and human<sup>17,21</sup>. As shown in Fig. 2, in cows LPS injection produced a significant rise in plasma glucose level at 0.5-1 hr, which was followed by hyperinsulinemia and slight hypoglycemia. Despite such changes in plasma glucose and insulin, plasma leptin level was little changed. In addition to insulin, cortisol in the plasma from LPS-injected cows was rapidly increased and sustained at much higher levels than saline-injected control until 5 hr post-injection (Fig. 2). The rapid rise of plasma glucocorticoids after LPS administration is quite consistent with the previously reported results not only in rodents but also in live-stock animals including sheep and cows<sup>6,7</sup>. Since plasma ACTH level is also increased after LPS injection<sup>6</sup>, the increased cortisol would be due, at least in part, to

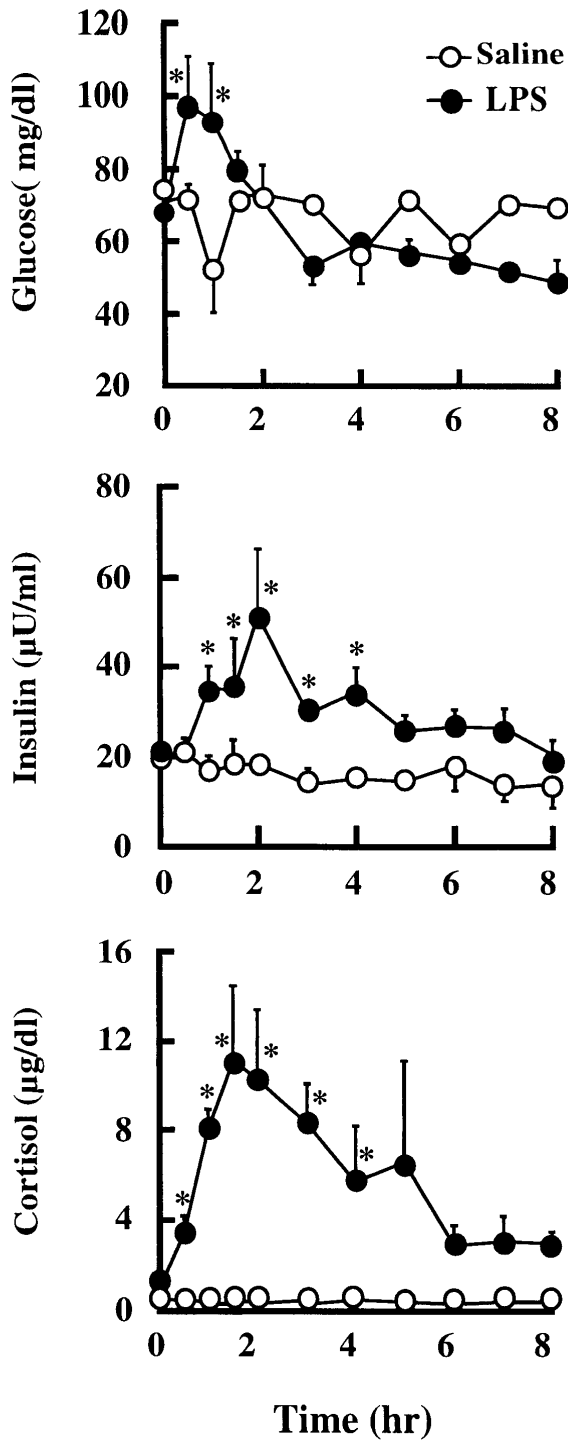


Fig. 2. Changes in plasma glucose, insulin and cortisol levels after LPS injection. LPS or saline was injected as in Fig. 1. Values are means  $\pm$  SEM for 6 cows for each treatment. \* $p < 0.05$  vs. saline control.

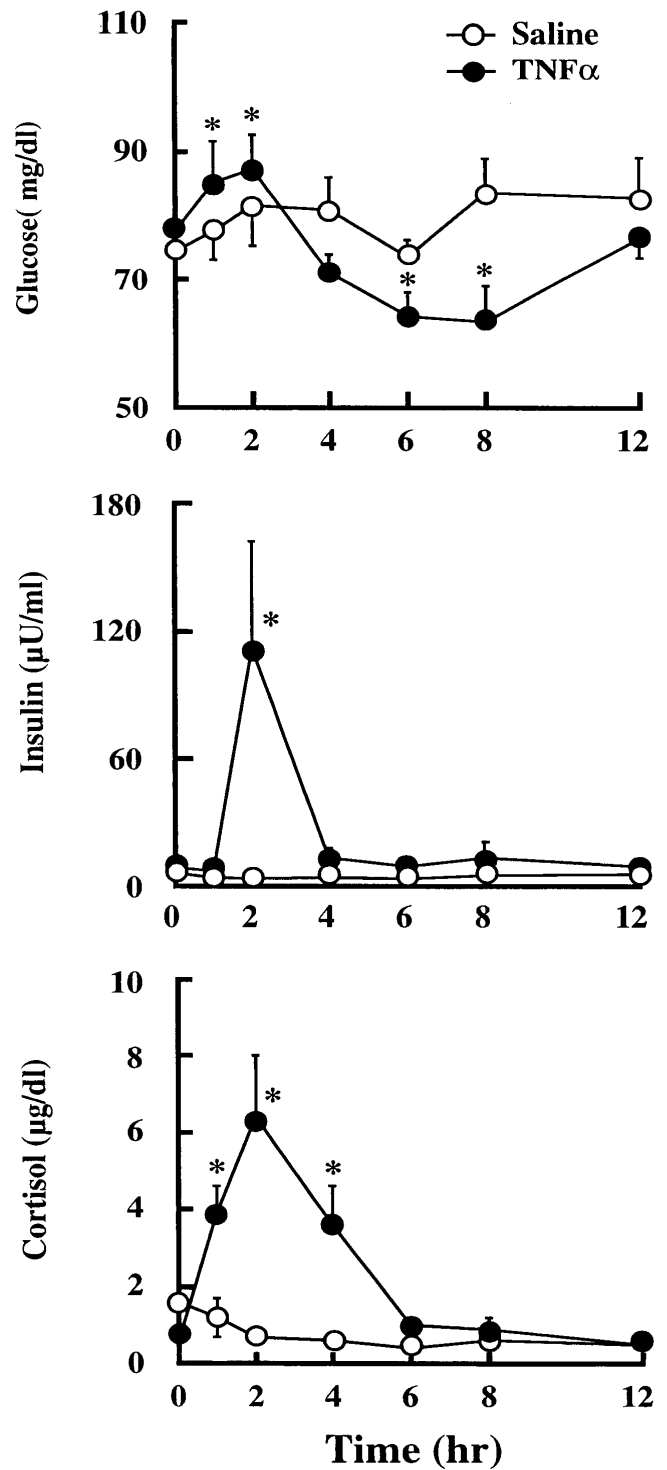


Fig. 3. Changes in plasma glucose, insulin and cortisol levels after TNF $\alpha$  injection. TNF $\alpha$  or saline was injected as in Fig. 1. Values are means  $\pm$  SEM for 6 cows for each treatment. \* $p < 0.05$  vs. saline

adrenocortical activation. However, such adrenocortical activation induced by LPS had no effect on plasma leptin level. Similar responses in plasma glucose, insulin and cortisol were also found after intravenous administration of TNF $\alpha$  (Fig. 3), while no noticeable change in plasma leptin was found (Fig. 1).

Thus, unlike in rodents and human, in cows no relation of plasma leptin level was found to plasma insulin and cortisol. These results are essentially the same as found in sheep<sup>19)</sup>, suggesting some regulatory mechanism and / or factors for leptin synthesis and secretion in ruminants different from those in rodents and human. Alternatively, it seems also possible that some suppressive factors, such as catecholamines, may cancel the stimulatory effects of insulin and glucocorticoids on leptin production. To discriminate these possibilities, it seems helpful to examine the effects of LPS and TNF $\alpha$  on plasma catecholamine levels. It is also needed to examine the direct effects of insulin, glucocorticoids and catecholamines on leptin synthesis in adipocytes of ruminant species and to compare them with those of rodents and human.

In summary, the present results showed no response of plasma leptin to LPS and TNF $\alpha$  administration in cows. These results are quite similar to our previous findings in sheep, collectively suggesting that leptin synthesis and secretion in ruminants are regulated by some mechanisms different from those in rodents and human. Furthermore, it is also unlikely, at least in ruminants, that plasma leptin is involved in endotoxin-induced anorexia.

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