Effects of long-term administration of recombinant bovine tumor necrosis factor-α on glucose metabolism and growth hormone secretion in steers

Shiro Kushibiki, DVM; Koichi Hodate, DVM; Hiroyuki Shingu, DVM; Yasuko Ueda, DVM; Yasuyuki Mori, DVM; Takashi Itoh, DVM; Yuichi Yokomizo, DVM

Objective—To investigate the effects of long-term administration of recombinant bovine tumor necrosis factor-α (rbTNF) on plasma glucose and growth hormone concentrations, and to determine whether treatment with rbTNF causes insulin resistance in steers.

Animals—5 steers treated with rbTNF and 5 steers treated with saline (0.9% NaCl) solution (control).

Procedures—In experiment 1, rbTNF (5.0 µg/kg of body weight) or saline solution (5 ml) was administered SC daily for 12 days. Blood samples were obtained before treatment, and plasma was harvested for determination of glucose, insulin, and growth hormone (GH) concentrations. In experiment 2, insulin, glucose, or growth hormone-releasing hormone (GHRH) was administered IV on days 7, 9, and 11, respectively, after initiation of rbTNF or saline treatment in experiment 1. Plasma glucose and insulin concentrations were measured before and at various times for 4 hours after insulin or glucose administration. Plasma GH concentrations were measured at various times for 3 hours after GHRH administration. Results—In experiment 1, administration of rbTNF resulted in hyperinsulinemia without hypoglycemia and decreased plasma GH concentrations. In experiment 2, plasma glucose concentrations were higher in steers treated with rbTNF and insulin than in controls. Plasma GH concentrations were lower in steers treated with rbTNF and GHRH than in controls.

Conclusions and Clinical Relevance—Prolonged treatment with rbTNF induced insulin resistance and inhibited GHRH-stimulated release of GH in steers. Results indicate that rbTNF is a proximal mediator of insulin resistance and inhibits release of GH during periods of endotoxemia or infection. (Am J Vet Res 2001;62:794–798)

Tumor necrosis factor-α (TNF), a potent cytokine produced primarily by activated macrophages, has multiple biological functions. In addition to antitumor activity, TNF has been associated with the metabolic and hormonal alterations observed in various states of enhanced catabolism, such as septic shock, endotoxemia, and acute infections.

In ruminants, septicemia or infection induces several disturbances in carbohydrate metabolism that are time-dependent. Administration of endotoxin to cattle induces an initial state of hyperglycemia, which is followed by profound hypoglycemia. Also, it has been reported that rats treated with endotoxin undergo transient hyperglycemia, although plasma glucose concentrations are not biphasic. In humans and rats, a correlation has been observed between TNF production and insulin resistance. However, in ruminants, a relationship has not been established between excessive TNF production and insulin resistance in the aforementioned pathologic conditions.

Indeed, administration of recombinant TNF to humans and animals induces some alterations in glucose metabolism, similar to alterations during septicemia, endotoxemia and acute infections, which suggests excessive TNF can lead to an insulin-resistant state. In calves, a single administration of recombinant bovine TNF (rbTNF) induces the same alterations in glucose metabolism that are observed during endotoxemia. We have previously reported that a single IV injection of rbTNF to heifers decreases glucose uptake in the early phase. In addition, long-term administration of TNF also results in insulin resistance in healthy rats. Previous research in calves indicated that prolonged administration of rbTNF will induce an increase in body temperature, decrease in total WBC count, and a decrease in plasma zinc concentrations. However, there have been few studies that investigate glucose metabolism after repeated administration of TNF. In addition, definitive studies on whether long-term administration of TNF impairs the actions of insulin in vivo are lacking.

In cattle, a substantial reduction in plasma growth hormone (GH) concentration accompanies the acute response of hepatic proteins. A similar decrease in plasma GH concentration has been observed in cattle that received lipopolysaccharide (LPS); administration of rbTNF also induced decreased plasma GH concentrations. Results of these studies suggest that increased production of TNF (as a mediator of the LPS response induced by enhanced catabolism) alters GH secretion in cattle. In another study performed in vitro, TNF receptors were found in the pituitary gland,
and TNF inhibited GHRH-stimulated release of GH from cultured bovine pituitary gland cells.\textsuperscript{12} The purposes of the study reported here were to determine whether prolonged administration of rbTNF causes insulin resistance, and to clarify the in vivo effects of long-term administration of rbTNF on insulin action, glucose-induced insulin secretion, and GHRH-stimulated release of GH in steers.

Materials and Methods

Cattle—For experiments 1 and 2, 10 Holstein steers between 9 and 11 months of age and weighing between 302 and 345 kg were used. Steers were housed individually in a mechanically ventilated barn. They were fed a diet of grass hay, alfalfa hay cubes, and grain twice daily (8 to 10 AM and 4 PM) and had access to water and mineral blocks ad libitum. All procedures were approved by our institutional Animal Care and Use Committee.

Experimental design—Steers were randomly assigned to 2 groups; 1 group received rbTNF, and the other group received saline (0.9% NaCl) solution (controls). Highly purified rbTNF\textsuperscript{a} was used. The rbTNF was produced using the Bacillus brevis host-vector system. Bacillus brevis is a useful host for efficient extracellular production of heterologous proteins.\textsuperscript{13} An expression-secretion plasmid for rbTNF was constructed and introduced into B brevis organisms by electroporation. The transformant carrying the rbTNF expression-secretion plasmid produced approximately 0.2 g of rbTNF/L extracellularly. The rbTNF was obtained from the culture supernatant and had proliferative activity against mouse Wehi 164 cells. Purified rbTNF was used for both experiments.

In experiment 1, the effects of daily administration of rbTNF on plasma glucose, insulin, and GH concentrations were determined. The rbTNF (3 µg/kg of body weight/day) or saline solution (5 ml/head/d) was injected SC at 11:30 AM daily for 12 days. Jugular venipuncture was performed on each steer, and blood samples (5 ml) were collected into heparinized tubes\textsuperscript{a} at 11:15 AM daily during the period of –2 to 15 days after the initiation of treatment. Blood samples were centrifuged immediately (4 C) for 25 minutes at 1,600 \( X \) g, and plasma was harvested and stored at –20 C until analyzed.

In experiment 2, we determined the effects of long-term rbTNF treatment on insulin-mediated glucose metabolism (trial 1), glucose-induced insulin secretion (trial 2), and GHRH-stimulated GH release (trial 3). Each trial was performed for both groups of steers at 7, 9, and 11 days, respectively, after initiation of rbTNF or saline treatment in experiment 1. A catheter\textsuperscript{b} was inserted in the left jugular vein of each steer at 10:00 AM, and was maintained by flushing with heparinized saline solution. Treatments for each trial were as follows: trial 1, IV injection of bovine insulin\textsuperscript{c} (0.2 U/kg); trial 2, IV injection of glucose\textsuperscript{d} (112.5 mg/kg); and trial 3, IV injection of GHRH\textsuperscript{e} (0.25 µg/kg). These preparations were injected via the catheter immediately following the rbTNF or saline treatment at 11:30 AM in experiment 1. Blood samples (4 ml) were collected from the catheter into heparinized tubes at –15, 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes after each injection. Samples were cooled on ice after collection. Within 2 hours of collection, samples were centrifuged (25 minutes at 1,600 \( X \) g) at 4 C, and plasma was harvested. Plasma samples were then frozen and stored at –20 C until analyzed.

Assays—Plasma glucose concentrations were determined by use of the glucose oxidase method.\textsuperscript{1} Plasma insulin concentrations were measured by use of a commercially available radioimmunoassay (RIA) kit.\textsuperscript{1} Plasma GH concentrations were determined by use of a double-antibody RIA procedure, as described elsewhere.\textsuperscript{1} Intra-assay and interassay coefficients of variation of the insulin and GH RIA were 3, 7, 6, and 9%, respectively. Lower limits of detection were 2.5 µU/ml for insulin and 1.0 ng/ml for GH.

Statistical analyses—In both experiments, all data were analyzed by use of 2-way repeated-measures ANOVA.\textsuperscript{1} When the effect of treatment or treatment \( \times \) time was found to be significant (\( P < 0.05 \)), differences among means were determined by use of the Fisher protected least significant difference post hoc test. Data are reported as mean ± SEM.

Results

Experiment 1—Concentrations of plasma glucose and insulin in steers of both groups were similar before initiation of treatment. Although plasma glucose concentrations of steers treated with rbTNF were significantly increased on day 1, glucose concentrations of steers in this group were similar to those of the control group from days 2 through 12. In contrast, plasma insulin concentrations in the steers treated with rbTNF were significantly higher than those in the control group from days 1 through 12 (Fig 1).

Prolonged treatment with rbTNF decreased plasma
GH concentration during and after the treatment period. Significant differences were detected between GH concentrations of steers in the rbTNF group and the control group on days 6, 10, and 13 (Fig 2).

Experiment 2—In trial 1, IV administration of insulin induced a hypoglycemic response in both groups (Fig 3). In steers treated with rbTNF, glucose concentrations were higher than those in the control group at 60 through 150 minutes after treatment. Plasma glucose concentration in the treated group returned to the baseline value at 150 minutes after insulin administration, whereas the control group, the baseline value was not reached until 210 minutes. In trial 2, changes in plasma glucose concentrations after IV glucose administration in both groups were similar throughout the sampling period; significant differences were not detected between groups. In contrast, the mean baseline insulin concentration in steers treated with rbTNF was higher than in the control group (Fig 4). Although plasma insulin concentrations increased in both groups after administration of glucose, concentrations remained higher in steers treated with rbTNF than in controls, indicating there was a notable difference in insulin secretion between groups. Insulin concentrations in both groups returned to baseline values at 60 and 45 minutes after treatment, respectively. However, the mean plasma insulin concentration in steers treated with rbTNF was significantly higher at 120 minutes after treatment, compared with the baseline value.

Mean baseline plasma GH concentrations before the initiation of trial 3 were similar in both groups (Fig 5) and reached peak values at 10 minutes after GHRH administration. However, the mean GH concentration in the treated group was significantly lower than that for the control group between 5 and 30 minutes after GHRH administration.

Discussion

We found that daily administration of rbTNF to steers induces hyperinsulinemia without hypoglycemia, indicating reduced insulin sensitivity. To further investigate the potential role of TNF in insulin resistance, we evaluated the effects of rbTNF on insulin-mediated glucose uptake. Steers treated with rbTNF had higher plasma glucose concentrations after administration of insulin than control steers, which indicates that rbTNF causes reduced actions of insulin. Our results were in accordance with results of studies performed in humans and other animals, which indicate that TNF is associated with insulin resistance. In rats, chronic TNF infusion can lead to whole-body insulin resistance. We also recently demonstrated that a single administration of rbTNF to heifers can cause hyperinsulinemia without hypoglycemia from 12 to 24 hours after the treatment. However, in the present study, plasma glucose concentration significantly increased only on the first day after treatment with rbTNF. In the early phase of infection or septicemia there is hepatic glycogenolysis and gluconeogenesis. Under these conditions, the rate of hepatic glucose output exceeds that of glucose removal by peripheral tissues, resulting in hyperglycemia.
Although TNF is primarily secreted from macrophages and has a major role in mediating inflammatory responses, it is also produced in muscle and adipose tissues and can influence glucose metabolism. High plasma TNF concentrations in rats may induce both hepatic and peripheral insulin resistance. Some mechanisms by which TNF might cause insulin resistance have been suggested in studies in which cultured cells were used. In adipocytes, administration of TNF resulted in downregulation of insulin-regulated glucose transporters. In addition, it has been reported that TNF impairs the insulin signaling pathway in hepatocytes and adipocytes. Impairments of insulin signaling are observed as reductions of insulin effects on receptor autophosphorylation and insulin receptor substrate phosphorylation. Alternatively, although muscle tissues are the primary site of glucose consumption, especially in response to insulin, the effects of TNF on the action of insulin in skeletal muscle are not well-known. It has been demonstrated that both acute and prolonged treatment with TNF increases glucose uptake in cultured human skeletal muscle cells. In rats, administration of TNF induces hypoglycemia, indicating increased glucose uptake into skeletal muscle. One recent study reported that TNF failed to affect glucose metabolism in cultured rat skeletal muscle cells. Furthermore, in another study, TNF impeded glycogenesis in cultured rat skeletal muscle cells. It has also been reported that administration of TNF causes insulin resistance in vivo, which was evidenced by an impaired ability of insulin to suppress hepatic glycogenesis and stimulate peripheral glucose uptake. It appears likely that the TNF-induced decrease of glucose disposal throughout the body during hyperinsulinemia is attributable to a smaller increment of insulin-stimulated glucose uptake by skeletal muscle.

In the present study, prolonged treatment with rbTNF increased insulin secretion, whereas it did not affect plasma glucose concentration after glucose administration. It has been reported that TNF in humans and rats interferes with insulin secretion from cultured B cells in response to glucose. The mechanism by which TNF inhibits glucose-induced insulin secretion is not clear. However, B-cell destruction induced by TNF has been well documented. A recent report suggested that TNF inhibits insulin secretion in human islets of Langerhans in vitro by increasing the output of nitric oxide. However, our in vivo observations contradict these reported in vitro effects. Although these discrepancies cannot yet be explained, it is possible that the increased insulin response to glucose after administration of rbTNF may be a compensatory function for the overall reduced action of insulin throughout the body.

In the study reported here, daily administration of rbTNF to steers resulted in decreased plasma GH concentration and GHRH-stimulated GH secretion. Previous studies have revealed that a single IV administration of rbTNF causes a decrease of the baseline GH concentration in calves. A recent report indicated that there are species differences in GH responses to LPS. Administration of LPS in humans and sheep causes an increase in plasma GH concentration, whereas administration of LPS in rats decreases plasma GH concentration. It has been suggested that TNF mediates the effects of LPS on in vivo and in vitro GH release in cattle.

Results of our study revealed that long-term administration of rbTNF induces insulin resistance in cattle. Also, administration of rbTNF considerably inhibits release of GH in steers. Results indicated that rbTNF is a proximal mediator of insulin resistance and most likely inhibits release of GH during periods of endotoxemia or infection.

References

15. Miles PDG, Romeo OM, Higo K, et al. TNF-induced