

# Leptin, tumor necrosis factor- $\alpha$ (TNF), and CD14 in ovine adipose tissue and changes in circulating TNF in lean and fat sheep<sup>2</sup>

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**ABSTRACT:** Four studies were designed to determine whether 1) tumor necrosis factor- $\alpha$  (TNF) and the Lipopolysaccharide (LPS) binding ligand, CD14, are produced by sheep adipose tissue; 2) nutritional reserves and/or short-term fasting affect circulating concentrations of TNF; 3) there is a relationship between TNF and metabolic factors in sheep; and 4) inflammation alters circulating concentrations of leptin. In Exp. 1 and 2, ewes were assigned, based on ultrasonic assessments of last-rib subcutaneous fat measurements to fat (fat thickness >1 cm; mean =  $1.52 \pm 0.03$  cm) or thin (fat thickness <1 cm; mean =  $0.25 \pm 0.03$  cm) groups. Fat and thin ewes were assigned to fed or fasted groups for a total of four groups (fed-fat; fasted-fat; fed-thin; fasted-thin). Fed-ewes had ad libitum access to feed, and fasted-ewes were prohibited feed 48 h before initiation of sample collection. In Exp. 1, subcutaneous fat samples were collected from just above the last rib for detection of TNF and CD14 mRNA, and immunoreactivity. Tumor necrosis factor- $\alpha$ -like immunoreactivity in adipocytes was sparse, more pronounced in cells in fed-ewes than fasted-ewes, and localized to membranes

between adjacent cells in nucleated regions. Immunoreactivity for CD14 was minimally observed but present in adipocytes and widely expressed in infiltrating monocytes and epithelial vascular cells. Leptin was detected in adipocytes. In Exp. 2, plasma samples collected every 6 h for 24 h were analyzed for plasma concentrations of TNF. Fat ewes had greater plasma concentrations of TNF than thin ewes ( $P = 0.039$ ). In Exp. 3, wethers were injected i.v. with interleukin- $1\beta$  or TNF. Blood samples were collected every 15 min for 8 h following injection. Plasma concentration of leptin was not affected by treatment ( $P > 0.39$ ). In Exp. 4, wethers were injected with LPS. Blood samples were collected every 15 min for 8 h following injection. Plasma concentration of leptin was not altered by LPS ( $P > 0.20$ ). These results provide evidence: 1) of TNF-like immunoreactivity within fat tissue; 2) that elements within fatty tissues have CD14 that may allow adipocyte function to be directly affected by LPS; 3) that plasma concentrations of leptin are not altered by LPS treatment; and 4) that circulating concentrations of TNF are elevated with obesity in sheep.

Key Words: Fat, Leptin, Lipopolysaccharides, Receptors, Sheep, Tumor necrosis factor.

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## Introduction

Adipose tissue was once viewed solely as an energy storage organ; however, recent evidence suggests a much more active role for adipocytes (Frühbeck et al., 2001). Adipocytes were reported recently to produce a number of factors capable of endocrine or paracrine actions. In humans, tumor necrosis factor- $\alpha$  (TNF) is

expressed in adipose tissue, and its expression increases with obesity (Hotamisligil et al., 1993; Hotamisligil et al., 1995; Kern et al., 1995). Several investigators have also reported that as adiposity increased in rodents and humans, peripheral concentrations of TNF increased (Hotamisligil et al., 1993; Corica et al., 1999; Tsukui et al., 2000), yet others failed to detect similar changes (Hotamisligil et al., 1995; Sewter et al., 1999; Kern et al., 2001). Considerable focus has now been given to the production of TNF by adipocytes because TNF is thought to induce insulin resistance and contribute to altered metabolism in obese humans and animals (Hotamisligil et al., 1993; Hotamisligil et al., 1995; Kern et al., 1995).

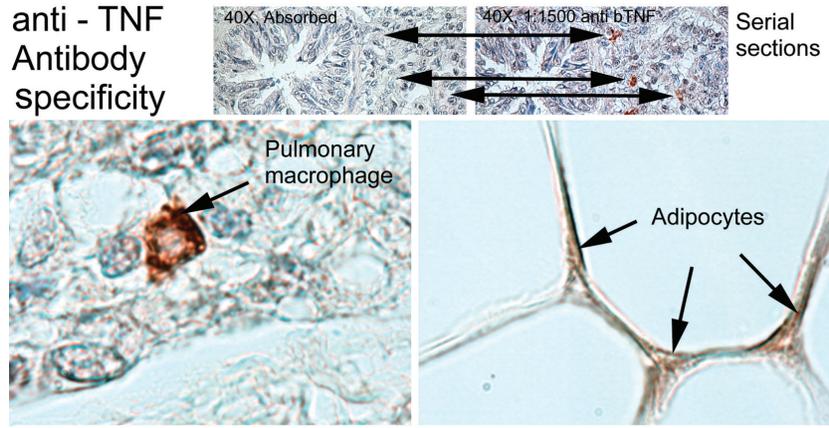
Lipopolysaccharide (LPS) has been used to provoke the release of TNF from human adipose tissue and

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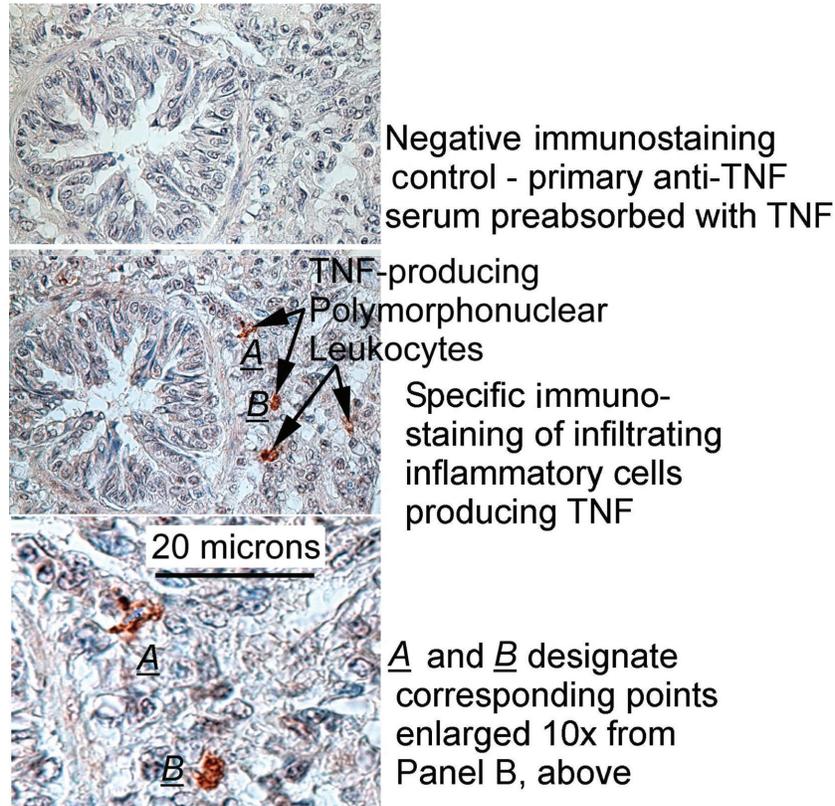
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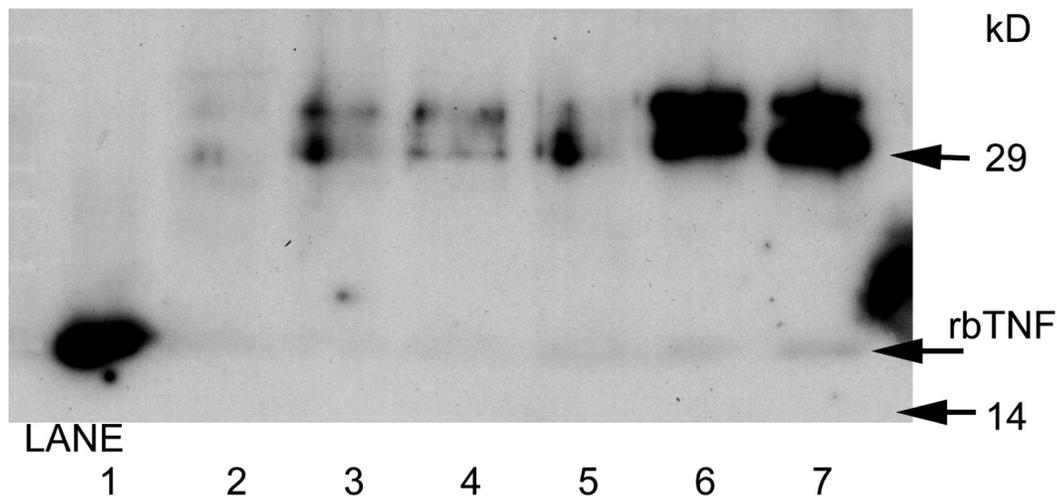
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**Figure 1.** Bovine lung tissue used as the positive and antibody-absorbed negative control for the adipose tissue staining for tumor necrosis factor- $\alpha$  (TNF) in Exp. 1. This tissue block is routinely used in our TNF immunostaining and presented because we know that monocytes in this slide should stain positively for TNF and negative when the antibody is absorbed. More importantly, under the microscope the tissue block is used to monitor the reaction of the antibody-treated slides with the diaminobenzidine (DAB) reporter to establish the exact time that the proper level of DAB deposition and staining has occurred. This minimizes artifacts associated with lack of detection because of improper underreaction with the DAB and artifacts of false-positive staining due to nonspecific reactions. This further confirms the specificity of the reactions.



**Figure 2.** Immunostaining of archived samples of lung from endotoxin-challenged lambs shows the specificity of the rabbit anti-bovine tumor necrosis factor- $\alpha$  (TNF) antibody. Infiltrating polymorphonuclear leukocytes are drawn to the lung early during the proinflammatory response by chemokines and activated to produce TNF. When the rabbit anti-bovine TNF serum is preincubated for 4 h at 37°C with 250 ng/mL recombinant bovine TNF, immunostaining within the infiltrating cells is competitively blocked.



**Figure 3.** The presence of tumor necrosis factor- $\alpha$  (TNF) in adipocytes was confirmed by Western blot and the identification of a 17.5-kDa band and the larger molecular weight, 29 kDa, variant of TNF recognized by rabbit anti-bovine TNF serum and visualized with a  $^{125}\text{I}$  donkey anti-rabbit IgG and autoradiography on film in Exp. 1. Lane 1 is recombinant bovine TNF, Lanes 2, 3, and 4 are from thin ewes, and Lanes 5, 6, and 7 are from fat ewes.

isolated adipocytes (Sewter et al., 1999), and presence of messenger RNA (mRNA) for the LPS receptor, CD14, was detected in isolated human preadipocytes and adipocytes (Sewter et al., 1999). Furthermore, in vivo treatments of LPS elevate peripheral concentrations of leptin (mice: Finck et al., 1998; rats: Roelfsema et al., 2001; humans: Landman et al., 2001). To date, adipose expression of TNF and CD14 and the effects of LPS and cytokines on plasma leptin concentrations have not been investigated in ruminants. Therefore, this study was designed to determine whether 1) TNF and CD14 are produced by sheep adipose tissue; 2) nutritional reserves and/or short-term fasting affect circulating concentrations of TNF; 3) there is a relationship between TNF and metabolic factors in sheep; and 4) inflammation alters circulating concentrations of leptin.

## Materials and Methods

### Animals

Before initiation of Exp. 1 and 2, all ewes (mature, nonlactating, blackface ewes) were individually housed under naturally occurring light conditions (February 25, 2000, through May 5, 2000) and provided ad libitum access to a concentrate feed (56% cracked corn, 25% cottonseed hulls, 10% soybean meal, 7% molasses, 1% limestone, 0.5% trace mineral salt, 0.2% dicalcium phosphate, 0.2% dynamate, 0.1% vitamin A, D, E cattle premix) beginning at least 1 wk prior to study. Ewes were classified as fat or thin, based on ultrasonic assessments of subcutaneous fat thickness over their last rib. Ewes with a fat thickness <1 cm were considered thin, and ewes with a fat thickness >1 cm were considered fat. Ewes were further

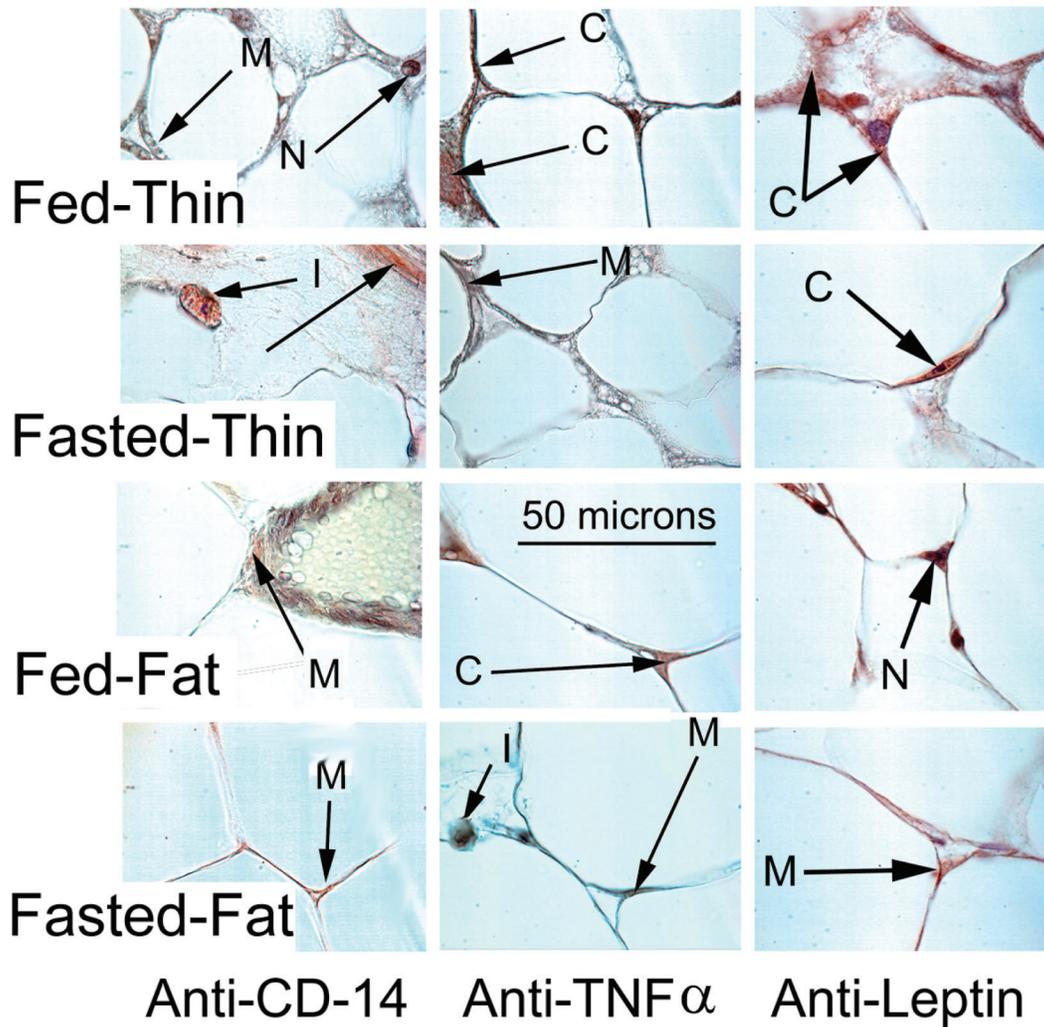
divided into ad libitum-fed vs. fasted groups for a total of four groups: fed-fat ( $111.8 \pm 2.1$  kg), fasted-fat ( $96.0 \pm 3.3$  kg), fed-thin ( $70.9 \pm 1.5$  kg), and fasted-thin ( $75.8 \pm 2.4$  kg). Fed-ewes had ad libitum access to feed throughout the experiment. Fasted-ewes were prohibited access to feed beginning 48 h prior to the initiation of sample collection. Water was available to all ewes at all times.

In Exp. 3 and 4, Hampshire-Suffolk wethers were housed under 12 h light:12 h dark lighting. Sheep were fed a total mixed ration at 3.5% of body weight and allowed ad libitum access to water. Sheep were acclimated to being tied with a halter for blood collection procedures.

### Experiment 1

Ewes (four per treatment) were manually restrained, and a small area just above the last rib was prepared for sterile incision. The area was anesthetized with lidocaine, and a 7-cm vertical incision was made. Two fat samples were removed and immediately frozen in liquid nitrogen. A third fat sample was removed and stored in Bouin's solution (250 mL of 37 to 40% formalin and 750 mL of 0.0715% picric acid and 0.05% glacial acetic acid).

**RNA Analysis.** Total RNA was isolated from fat tissue and analyzed for presence mRNA for TNF and CD14. The RNA was treated with DNase to remove any DNA contamination prior to reverse transcription (RT)-PCR. The presence of mRNA for TNF in the fat was confirmed via RT-PCR using primers (5'ATC AGC CGC ATT GCA GTC TCC TAC 3' and 5'GAT GGT TGG TGG CCC GTT GTC 3') for TNF. The presence of mRNA for CD14 in fat was confirmed via RT-PCR using primers (5'TAT TGG AGG GCC GGG AAC TTG



**Figure 4.** Immunostaining patterns for CD-14, tumor necrosis factor- $\alpha$  (TNF), and leptin in adipose tissue samples from thin and fat, fed and fasted ewes in Exp. 1. Arrows indicate locations of specific antigen presence. Key: C, cytoplasmic; M, membrane associated; N, nuclear; I infiltrating monocyte. All samples photographed at the same magnification; size and distances indicated by superimposed 50- $\mu$ m indicator bar.

3' and 5'CCA CCC TCG ACG GCA ACC ATA 3'). Products of RT-PCR were cloned into PCR2 vectors and submitted to the Auburn University sequencing facility to verify that bands observed on electrophoresis were indeed TNF and CD14 (GenBank Accession Numbers AY289202 and AY289201).

**Immunocytochemistry.** Fixed tissues were deparaffinized in xylene baths and dipped in absolute ethanol. Intrinsic peroxidase activity was eliminated by incubating slides in methanolic 3% hydrogen peroxide for 30 min. Samples were rehydrated through serially decreasing concentrations of ethanol to pure water and transferred to a Tris (0.01 M, pH 7.5)—saline (0.15 M) solution. Nonspecific binding of antibody was blocked by a 60-min incubation of slides in 3% normal goat serum. Primary antibodies (1:100 mouse anti-ovine CD14, Serotec Inc., Raleigh, NC, 1:2000 rabbit anti-bovine TNF, Kenison et al., 1990, 1:1700 rabbit anti-leptin, Richards et al., 2000) were diluted in 1%

normal goat serum and incubated with respective slides overnight at 4°C in a humidified chamber. Visualization of specific antigens in the cells was accomplished by the deposition of an oxidation product of diaminobenzidine in the presence of horseradish peroxidase as performed with an avidin-biotin complex (Vectastain, Vector Laboratories, Burlingame, CA) procedure. Bovine lung tissue used as the positive and antibody-absorbed negative control for the adipose tissue staining for TNF (Figure 1). This tissue block is routinely used in our TNF immunostaining and presented because it is known that monocytes in this slide should stain positively for TNF and negatively when the antibody is absorbed. More importantly, under the microscope, the tissue block is used to monitor the reaction of the antibody-treated slides with the diaminobenzidine reporter to establish the exact time that the proper level of diaminobenzidine deposition and staining has occurred. This minimizes artifacts associ-

ated with lack of detection because of improper under-reaction with the diaminobenzidine and artifacts of false-positive staining due to nonspecific reactions. This further confirms the specificity of the reactions. Cells were counterstained with Gill's hematoxylin formula no. 3 (0.6% hematoxylin, 0.06% sodium iodate, 10.6% aluminum sulphate, 6% glacial acetic acid, 25% ethylene glycol), and coverslips mounted with Fisher Permount. Sections were photographed under oil at 100X using a Polaroid DMC-3 camera with automatic tungsten light correction. Images were further compiled with Adobe Photoshop. Mean cell dimensions and diameter were measured in cells with a nuclear diameter of 5 to 6 microns  $\mu\text{m}$  (typical nuclear size), so that some degree of normalization can be established regarding cell slice is taken.

**Western Blot.** Validation of TNF presence in adipocytes was confirmed by Western blot using rabbit anti-bovine TNF serum. As TNF is produced as a membrane-bound protein, which is proteolytically released by TNF- $\alpha$  converting enzyme (Decker et al., 1987; Kriegler et al., 1988; Black et al., 1997), a membrane-enriched preparation of proteins from the fat tissue was utilized. The procedure identified a 17.5-kDa band when membrane proteins, isolated by percoll density centrifugation from adipocyte membranes (Belsham et al., 1980), were electrophoretically separated on polyacrylamide gel, blotted on nitrocellulose membranes, and probed with the antibody. Specific protein bands from the gels were visualized with  $^{125}\text{I}$  donkey anti-rabbit IgG and autoradiography on film.

#### Experiment 2

Ewes (five per treatment) used in this study were also described in a report detailing the diurnal pattern of leptin secretion (Daniel et al., 2002). Briefly, jugular blood samples were collected via indwelling jugular cannula every 15 min for 24 h (using red-filtered light during the dark period), beginning 48 h after initiation of feed restriction or the congruent interval in fed-ewes. Blood was immediately placed into EDTA-containing tubes and centrifuged at 4°C. Plasma samples were stored on ice until transported to the lab and stored at -20° or -80°C until assayed.

**Plasma Measurements.** Plasma measurements of TNF were determined in samples collected every 6 h in a single assay by RIA as described previously (Kenison et al., 1990). The minimum detectable TNF was 16 pg per tube, and the intraassay CV was 16%. Plasma concentrations of leptin were selected from data in another report (Daniel et al., 2002) to correspond to samples analyzed for TNF in this study. The leptin RIA was described previously (Delavaud et al., 2000). Samples were analyzed in duplicate aliquots of 200  $\mu\text{L}$  each in seven assays with inter- and intra-assay CV of 6% and 2%, respectively. Plasma concentrations of glucose and nonesterified fatty acids were determined in duplicate aliquots of samples collected every 6 h using commercially available kits (procedure No. 115; Sigma Diagnostics, St. Louis, MO, and NEFA

C; Wako Chemicals GmbH, Neuss, Germany, respectively). The inter- and intra assay CV were <17%.

#### Experiment 3

Wethers were fitted with an indwelling jugular cannula the day before the experiment for blood sample collection and delivery of interleukin-1 (IL-1), TNF, or saline (recombinant human (rh)IL-1 and rhTNF from PeproTech, Inc., Rocky Hill, NJ, USA; 3-mL sterile saline followed test substances). Blood samples were collected at 15 min intervals from -1 h to 8 h relative to injection (h = 0) of IL-1 (0.5 and 5  $\mu\text{g}/\text{kg}$ ), TNF (0.5 and 5  $\mu\text{g}/\text{kg}$ ), or saline. Core body temperatures were recorded hourly. A minimum of 1-wk recovery was allowed between replicates. Plasma was harvested and stored at -20°C until assayed for leptin.

#### Experiment 4

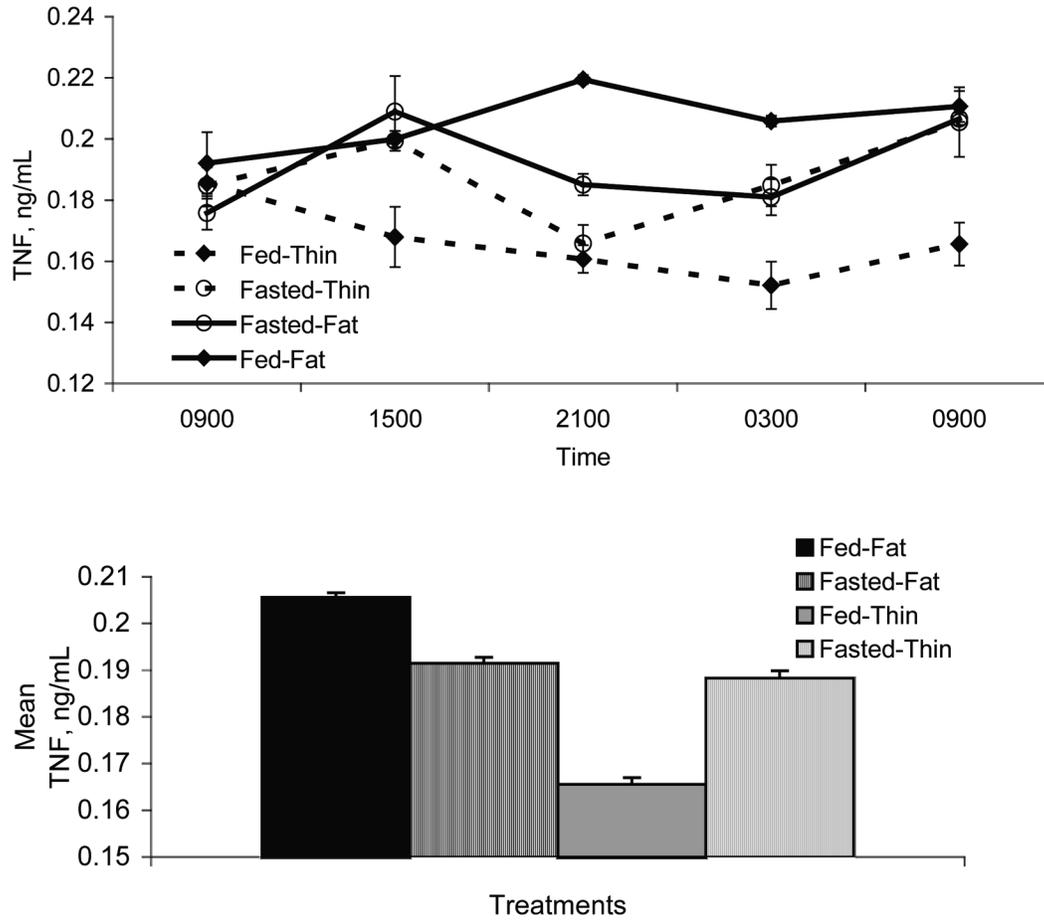
Wethers were fitted with an indwelling jugular cannula the day before the experiment for blood sample collection and delivery of LPS (0.6  $\mu\text{g}/\text{kg}$  of *E. coli* 055:B5 endotoxin; Sigma, St. Louis, MO, USA) or saline. Blood samples were collected at 15-min intervals from -1 h to 8 h relative to injection of LPS (h = 0). Core body temperatures were recorded hourly. A minimum 2-wk recovery interval was allowed between replicates. Plasma was harvested and stored at -20°C until assayed for leptin.

#### Statistics

All statistical analysis was performed using SAS (SAS Inst. Inc., Cary, NC). Correlations were determined between fat thickness and values for leptin mRNA expression, mean TNF, glucose, and free fatty acid concentrations in both fat and thin ewes. Correlations were also determined between glucose, TNF, NEFA, and 6-h leptin values. Effect of feed-treatment, body condition, time, and interactions on plasma concentrations of TNF, glucose, and free fatty acids were determined using GLM procedures for repeated measures with animal within feed-treatment by body condition used as the error term. For Exp. 3 and 4, leptin mean concentration, area under the curve, number of pulses, pulse amplitude, and nadir was determined using CLUSTER analysis (Veldhuis and Johnson, 1986). In Exp. 3, effects of cytokine treatment on leptin mean plasma concentrations, area under the curve, number of pulses, pulse amplitude, and nadir were determined using GLM procedures. In Exp. 4, effects of LPS treatment on leptin mean plasma concentrations, area under the curve, number of pulses, pulse amplitude, and nadir were determined using GLM procedures. Least squares means separation procedures were used when the effect was significant.

#### Results

Specific staining to cells that have TNF-immunoreactivity was observed in polymorphonuclear leuko-



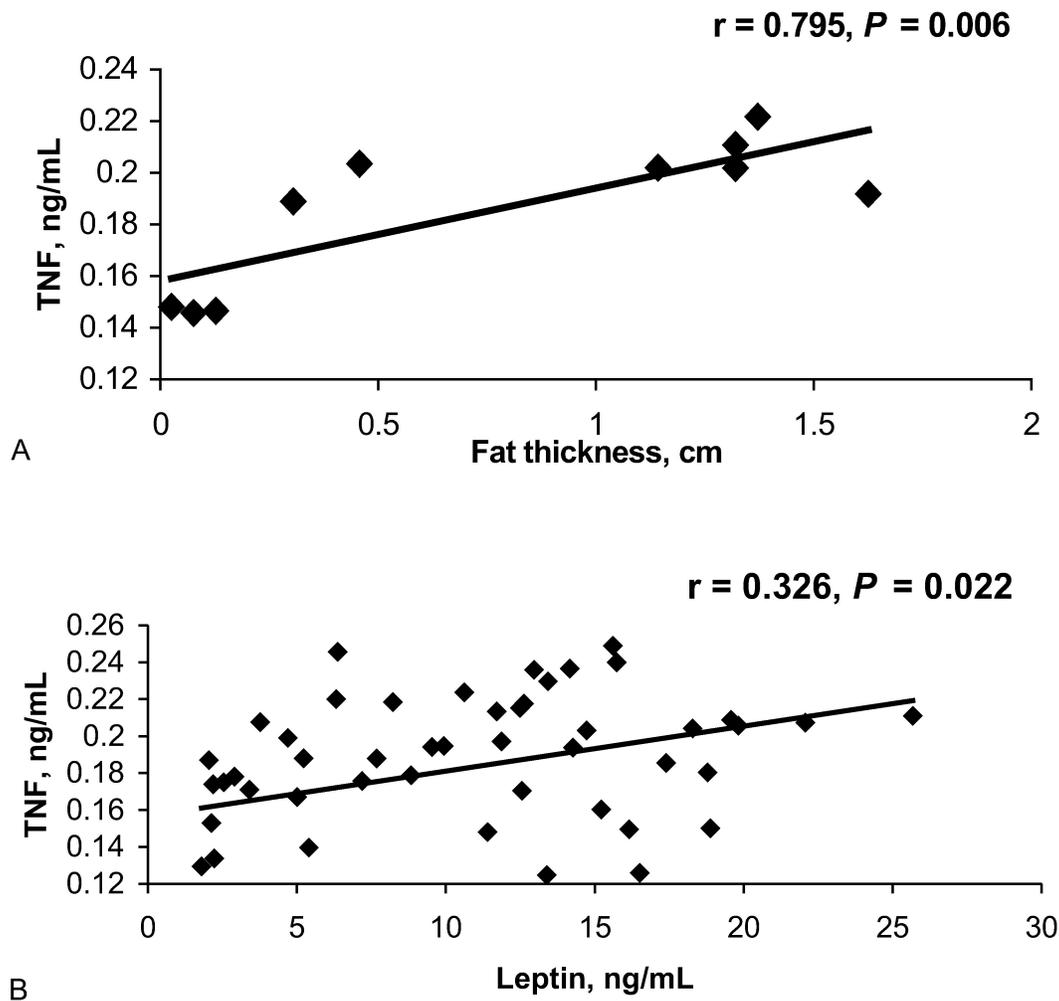
**Figure 5.** Fat ewes had greater plasma concentrations of tumor necrosis factor- $\alpha$  (TNF) than thin ewes ( $P = 0.039$ ), and there was a tendency for a treatment by body condition interaction ( $P = 0.077$ ), such that fed-fat ewes had greater plasma concentrations of TNF than fed-thin ewes ( $P = 0.001$ ), but plasma concentrations of TNF did not differ between fasted-fat and fasted-thin ewes ( $P = 0.71$ ) in Exp. 2.

cytes in lung tissue from endotoxin-challenged lambs (Figure 2). Staining in the lung was eliminated when the antibody was preadsorbed to block binding by incubation with  $1 \mu\text{M}$  recombinant bovine TNF (Figure 1b). Western blot of membrane proteins isolated from ewe fat confirmed the presence of TNF in adipocytes (Figure 3). Clean, membrane-associated TNF reactivity was observed in fat from sheep (Figures 1 and 4). Cells staining positive for TNF included infiltrating monocytes, some vascular cells, and adipocytes (Figure 4 and data not shown). Tumor necrosis factor- $\alpha$ -like immunoreactivity in adipocytes was sparse, appeared more pronounced in cells in fed-ewes than fasted-ewes, and was localized to the membrane in nucleated regions. The presence of TNF appeared to be more related to cell size than feed-treatment, although TNF presence may be related to some aspect of the cell dynamics of volume change as affected by feed-treatment (i.e., shrinking vs. enlarging or some aspect of the transition). Immunoreactivity for CD14 was minimally observed but present in adipocytes and widely expressed in infiltrating monocytes and epithelial vascular cells (Figure 4). Leptin was localized uni-

formly in the cytoplasm associated with the membrane (Figure 4). Overall, thin ewes had smaller adipocytes than fat ewes (cell diameter: fed-fat  $74.2 \pm 12 \mu\text{m}$ , fasted-fat  $70.4 \pm 8 \mu\text{m}$ , fed-thin  $54.3 \pm 4 \mu\text{m}$ , fasted-thin  $50.7 \pm 3 \mu\text{m}$ ; fat vs. thin  $P < 0.03$ ).

Reverse transcription-polymerase chain reaction of fat RNA revealed the expression of TNF and CD14 in fat tissue. The region of the TNF mRNA cloned was 99% homologous with ovine TNF (GenBank accession number X55152). The region of the CD14 mRNA cloned was 96% homologous with bovine CD14 (GenBank Accession Number D84509). The presence of mRNA for TNF and CD14 mRNA in fat tissue was not detectable by Northern Blot (not shown).

There was no effect of fasting ( $P = 0.7$ ) or time ( $P = 0.3$ ) on plasma concentrations of TNF. Plasma concentrations of TNF were greater in fat ewes than thin ewes ( $P = 0.039$ ; Figure 5), and there was a tendency for a treatment by body condition interaction ( $P = 0.077$ ), such that fed-fat ewes had greater plasma concentrations TNF than fed-thin-ewes ( $P = 0.001$ ), but plasma concentrations of TNF did not differ between fasted-fat and fasted-thin-ewes ( $P = 0.70$ ).



**Figure 6.** Correlation of mean plasma concentrations of tumor necrosis factor- $\alpha$  (TNF) with fat thickness in fed-ewes (A) and correlation of mean plasma concentrations of TNF with leptin in fed-ewes (B) in Exp. 2.

Effects of feed-treatment on plasma concentrations of leptin and leptin profiles were reported in Daniel et al. (2002), and the plasma used in this study was from the same sheep. Plasma concentrations of glucose (fed  $69.8 \pm 1.1$  vs. fasted  $63.3 \pm 1.7$  mg/dL,  $P = 0.035$ ) and free fatty acids (fed  $0.08 \pm 0.01$  vs. fasted  $0.57 \pm 0.02$  mEq/L,  $P = 0.001$ ) were significantly affected by feed-treatment. Plasma concentrations of free fatty acids were also affected by time ( $P = 0.016$ ), such that plasma concentrations of free fatty acids at 24 h after the initiation of blood sampling was greater than at any other time studied. All other time points did not differ. Additionally, the feed-treatment by time interaction was significant, such that the plasma concentrations of free fatty acids at 24 h after the initiation of blood sampling was significantly greater in fasted-ewes than at any other time point ( $P \leq 0.001$ ) and did not differ within fed-ewes ( $P \geq 0.4$ ).

Fat thickness was correlated with mean plasma concentrations of TNF ( $r = 0.8$ ,  $P = 0.006$ ; Figure 6A) in fed-ewes. Leptin was correlated with TNF ( $r = 0.33$ ,  $P = 0.02$ ; Figure 6B) in fed-ewes.

Treatment of wethers with LPS or the inflammatory cytokines, IL-1, and TNF invoked a febrile response but did not alter circulating concentrations of leptin (Tables 1 and 2). There was a tendency for LPS treatment to increase the leptin peak height relative to saline-treated wethers ( $P = 0.07$ ).

## Discussion

The procedures of RT-PCR and immunocytochemistry provide evidence that TNF and CD14 are both produced by subcutaneous adipocytes in sheep. Previous research has reported the presence of TNF in human adipose tissue (Hotamisligil et al., 1995; Sewter et al., 1999). The presence of TNF protein appears to be more related to cell size than feed-treatment as would be expected given that fat ewes had greater circulating concentrations of TNF than thin ewes but feed-treatment had no effect. Bertin et al. (2000) reported that plasma concentrations of TNF were not affected by changes in plasma glucose or intensive insulin treatments in type 2 diabetics. Other research-

**Table 1.** Effect of administration of the cytokines, interleukin-1 $\beta$ (IL-1) or tumor necrosis factor- $\alpha$ (TNF), on episodic patterns of circulating concentrations of leptin in Exp. 1<sup>a,b</sup>

	IL-1		Saline	TNF	
	0.5 $\mu$ g/kg	5 $\mu$ g/kg		0.5 $\mu$ g/kg	5 $\mu$ g/kg
Mean, ng/mL	2.8 $\pm$ 0.4	2.6 $\pm$ 0.4	2.3 $\pm$ 0.3	2.7 $\pm$ 0.3	3.6 $\pm$ 0.8
Area under the curve	1,363 $\pm$ 184	1,299 $\pm$ 162	1,039 $\pm$ 126	1,215 $\pm$ 138	1,637 $\pm$ 361
Number of peaks	2.8 $\pm$ 0.5	4.0 $\pm$ 0.4	3.0 $\pm$ 0.6	3.4 $\pm$ 0.2	2.8 $\pm$ 0.6
Peak height, ng/mL	3.3 $\pm$ 0.4	3.4 $\pm$ 0.4	2.9 $\pm$ 0.2	3.3 $\pm$ 0.3	4.2 $\pm$ 0.9
Nadir, ng/mL	2.1 $\pm$ 0.4	1.6 $\pm$ 0.4	1.8 $\pm$ 0.2	1.9 $\pm$ 0.2	2.9 $\pm$ 0.7

<sup>a</sup>Data are presented as mean  $\pm$  SE.

<sup>b</sup>Treatments did not have a significant effect on any of the parameters examined.

ers have reported that elevated TNF in obese patients fell significantly with weight loss (Kern et al., 1995; Dandona et al., 1998); however, this reflected a long-term change in nutrient intake and not a short-term alteration as existed in the current study. Plasma concentrations of TNF were also correlated with fat thickness and leptin in fed-ewes, further providing evidence that the production of TNF by fat contributes to circulating concentrations of TNF in sheep. Some research groups also have detected elevated circulating TNF with obesity in humans (Corica et al., 1999; Tsukui et al., 2000), but others have not (Hotamisligil et al., 1995; Sewter et al., 1999; Kern et al., 2001). Perhaps extreme differences in body condition are necessary to detect elevated circulating TNF with obesity. Koistinen et al. (2000) observed a correlation between subcutaneous adipose tissue TNF mRNA levels and BMI that was only significant with the inclusion of five morbidly obese men. The increased circulating TNF with increased fat thickness indicates TNF may be acting as a signal of body condition or at the very least contributing to obesity-related disorders.

The role of TNF produced by fat tissue is unclear. In a study by Fawcett et al. (2000), TNF inhibited leptin release from cultured subcutaneous and omental adipocytes. Additionally, Bruun et al. (2002) reported that incubation of abdominal subcutaneous adipose tissue with TNF decreased leptin production and

gene expression. However, Zhang et al. (2000) reported that TNF decreased leptin mRNA expression and increased leptin release from cultured human adipocytes. Treatment of 3T3-L1 adipocytes and mice in vivo also stimulated increased secretion of leptin (Kirchgessner et al., 1997), and in vitro stimulation of primary murine adipocytes with TNF also increased production of leptin (Finck et al., 1998). In the current study however, peripheral administration of TNF did not alter circulating concentrations of leptin. Thus, TNF may be acting in a paracrine manner on adipocytes.

Tumor necrosis factor- $\alpha$  may also be acting in an endocrine manner. In sheep, i.v. injection of TNF resulted in increased circulating concentrations of GH (Daniel et al., 2001). Tumor necrosis factor- $\alpha$  treatments also transiently increased GH secretion in heifers, although TNF inhibited GHRH-induced GH secretion (Kushibiki et al., 2000 and 2001a). Additionally, TNF inhibited GHRH-stimulated GH release in cultured bovine and ovine pituitaries (Elsasser et al., 1991; Fry et al., 1998).

Tumor necrosis factor- $\alpha$  can also alter metabolism and feed intake. Fantino and Wieteska (1993) reported that central administration of low doses of TNF inhibited feed intake but did not induce deleterious effects. Administration of TNF also promoted insulin resistance in steers (Kushibiki et al., 2001a, b). In cattle, TNF administration was followed by an initial increase in circulating concentrations of glucose followed by prolonged hypoglycemia (Kenison et al., 1991; Kushibiki et al., 2000). Tumor necrosis factor- $\alpha$  administration also caused an initial increase in triglycerides followed by depressed circulating concentrations of triglycerides in heifers (Kushibiki et al., 2000). Others, however, have observed that triglycerides and NEFA were not affected by TNF treatment of bull calves (Kenison et al., 1991).

Tumor necrosis factor- $\alpha$  produced by adipose tissue may also be part of the inflammatory response to endotoxin. Sewter et al. (1999) observed that LPS stimulated increased TNF accumulation in the media from cultured human adipose tissue and isolated adipocytes. Furthermore, Sewter et al. (1999) observed the

**Table 2.** Effect of lipopolysaccharide (LPS; 0.6  $\mu$ g/kg) administration on episodic patterns of circulating concentrations of leptin in Exp. 2<sup>a</sup>

	LPS <sup>b</sup>	Saline
Mean, ng/mL	3.4 $\pm$ 1.7	3.8 $\pm$ 2.0
Area under the curve	1,457 $\pm$ 823	1,704 $\pm$ 882
Number of peaks	3.2 $\pm$ 1.3	4.0 $\pm$ 1.2
Peak height, ng/mL <sup>c</sup>	4.3 $\pm$ 1.8	4.2 $\pm$ 2.1
Nadir, ng/mL	2.9 $\pm$ 1.5	3.2 $\pm$ 1.8

<sup>a</sup>Data are presented as mean  $\pm$  SE.

<sup>b</sup>Treatment with LPS did not have a significant effect on any of the parameters examined.

<sup>c</sup>There was a tendency for treatment with LPS to alter peak height, such that LPS-treated sheep had a greater leptin peak height than saline-treated sheep ( $P = 0.069$ ).

presence of mRNA for CD14, the LPS “receptor,” in both human preadipocytes and adipocytes, suggesting that LPS may regulate adipocyte regulatory events. For example, in response to LPS administration, there is an increase in circulating concentrations of leptin (Finck et al., 1998; Landman et al., 2001; Roelfsema et al., 2001). However, in the current study, peripheral administration of neither LPS nor the inflammatory cytokines, TNF or IL-1, altered circulating concentrations of leptin, although all three did induce a febrile response. The failure to detect an increase in leptin in response to LPS administration could be due to species differences as Finck et al. (1998) utilized a mouse model. Alternatively, the 8-h post-LPS sample collection could have been insufficient to detect an LPS-induced increase in leptin, as increases in leptin in response to LPS were observed 16 h after LPS administration in nonhuman primates and rats (Landman et al., 2001; Roelfsema et al., 2001). Furthermore, in women, peripheral concentrations of leptin were not altered 7 h after LPS challenge but were increased 24 h after LPS (Landman et al., 2001). As for additional evidence supporting an adipose tissue-immune relationship, humans with sepsis or septic shock have elevated peripheral concentrations of leptin (Arnalich et al., 1999). Additionally, *in vitro* stimulation of human adipocytes with LPS increased production of TNF (Sewter et al., 1999), and *in vitro* stimulation of primary murine adipocytes with TNF, but not LPS, increased production of leptin (Finck et al., 1998). These results indicate both an immune role for the adipose tissue and a paracrine function for TNF in adipose tissue.

### Implications

These results provide evidence that the lipopolysaccharide binding ligand, CD14, and tumor necrosis factor- $\alpha$  is produced by fat tissue in sheep and with other reports suggest that the fat tissue may have a role in immune responses. Additionally, circulating concentrations of tumor necrosis factor- $\alpha$  are greater in fat ewes, suggesting an additional mechanism exists for the fat cell to signal body condition.

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