Effects of systemic inflammation on insulin sensitivity in horses and inflammatory cytokine expression in adipose tissue

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Objective—To determine whether an inflammatory challenge induces insulin resistance in horses and examine possible contributions of adipose tissue to inflammatory cytokine production.

Animals—15 adult mares.

Procedures—Lipopolysaccharide (0.045 µg/kg, IV) or saline solution was administered, and insulin sensitivity was determined by means of the hyperinsulinemic, euglycemic clamp procedure or an adipose tissue biopsy was performed. Adipose tissue samples were collected, and mature adipocytes were obtained. Mature adipocytes were stimulated with lipopolysaccharide or dedifferentiated into preadipocytes and then stimulated with lipopolysaccharide. Interleukin-1, interleukin-6, and tumor necrosis factor α expression in blood, adipose tissue, and adipocytes was quantified with a real-time, reverse transcriptase–PCR assay.

Results—Lipopolysaccharide induced a transient increase in insulin sensitivity followed by a reduction in insulin sensitivity at 24 hours. Increased cytokine expression was observed in blood and adipose tissue following administration of lipopolysaccharide, and adipocytes and preadipocytes stimulated with lipopolysaccharide stained positive for tumor necrosis factor α. Expression of interleukin-1, interleukin-6, and tumor necrosis factor α was detected in preadipocytes stimulated with lipopolysaccharide, and interleukin-6 and tumor necrosis factor α were detected in mature adipocytes stimulated with lipopolysaccharide.

Conclusions and Clinical Relevance—Results indicated that insulin resistance develops following systemic inflammation in horses and suggested that adipose tissue may contribute to this inflammatory response. Methods to regulate insulin sensitivity may improve clinical outcome in critically ill patients. (Am J Vet Res 2008;69:130–139)

Obesity-related insulin resistance is a well-established phenomenon in horses, and is associated with a number of disorders, including altered reproductive function and laminitis. Although obesity-related insulin resistance likely involves many complex underlying interactions, a growing body of evidence points to both correlative and causative relationships between inflammation and insulin resistance, and obesity in humans is now described as a mild, chronic inflammatory state. Circulating concentrations of inflammatory molecules, including acute phase proteins and inflammatory cytokines such as TNFα, are high in obese humans, with higher concentrations seen in obese patients with insulin resistance and type II diabetes. Successful reversal of insulin resistance in humans by administering anti-inflammatory salicylates such as aspirin provides further evidence for the role of inflammation in insulin resistance. Furthermore, insulin resistance in humans can result from exposure to endotoxin, which causes acute systemic inflammation. Therefore it is reasonable to postulate that systemic inflammation may also induce insulin resistance in horses.

Several key molecules involved in the development of insulin resistance are produced by adipose tissue. This finding has spurred researchers to investigate the

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role of adipose tissue in the regulation of insulin sensitivity.

Adipose tissue was once considered only a reservoir for energy storage, but is now recognized as an endocrine organ with an active role in whole-body energy homeostasis. Adipose tissue is composed of MAs and stromal vascular cells such as fibroblasts, PAs, and tissue-resident macrophages. Macrophages are known to contribute to inflammation, but adipocytes can also have inflammatory properties. A number of inflammatory mediators that directly contribute to insulin resistance, such as IL-1, IL-6, and TNFα, are synthesized and secreted by adipose tissue. Thus, the importance of excess adipose tissue as a potentially important contributor to the development of insulin resistance should not be understated.

We have recently identified a significant association between obesity, insulin resistance, and increased concentrations of inflammatory cytokines in horses. This information, combined with previous associations between obesity, insulin resistance, and laminitis, suggests that there may be a link between inflammatory conditions and the occurrence of laminitis. Clinical situations involving factors such as obesity, sepsis, and postoperative conditions associated with inflammation where the potential for insulin resistance exists may, in fact, predispose horses to develop complications such as laminitis.

Administration of LPS is a commonly used method for inducing an acute, but transient, inflammatory response characterized by increases in heart rate, body temperature, respiratory rate, and circulating concentrations of inflammatory cytokines in many species. In particular, administration of LPS in horses increases circulating concentrations of TNFα. The present study was designed to test the hypothesis that an inflammatory response directly induces insulin resistance in horses and that adipose tissue actively participates in the inflammatory response in horses, contributing to the development of insulin resistance. Specifically, the purposes of the study reported here were to identify changes in insulin sensitivity and inflammatory cytokine mRNA expression in blood and adipose tissue after LPS administration in horses and to measure production of inflammatory cytokines by cultured equine adipocytes following stimulation with LPS.

Materials and Methods

Animals—All procedures were conducted in accordance with accepted standards of humane animal care; the experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

Mares of mixed light-horse breeds (6 to 19 years of age) randomly selected from the University of Kentucky's general research herd were used in the study. Experiments were conducted during the nonbreeding season, and mares were not pregnant at the time of the study. Mares in the research herd were maintained on pasture with ad libitum access to water and timothy hay (approx 7.5% crude protein content and approx 2.5% fat content as fed) throughout the study period.

Experiment 1—Five mares were used to determine changes in insulin sensitivity and cytokine expression in response to LPS administration. Mares ranged from 10 to 14 years old; body condition score, determined on a scale from 1 (emaciated) to 9 (extremely obese), ranged from 4 to 6.

In all mares, an indwelling catheter designed for long-term use was inserted into a jugular vein. Catheters remained in place for the duration of the experiment. Triple antibiotic ointment was applied to the catheter insertion site, and catheters were covered with a bandage when not in use. Food, but not water, was withheld for 14 hours prior to each part of the experiment.

Baseline rectal temperature, pulse rate, and respiratory rate were recorded, and saline solution (0.9% NaCl; 2 mL; day 1) or LPS* (0.045 µg/kg; day 4) was administered IV. Blood samples (2 mL) were collected into evacuated tubes prior to administration of saline solution or LPS and then hourly for 7 hours. Blood samples were stored at room temperature overnight and then at –20°C until total RNA could be isolated; total RNA was isolated with a commercially available kit used in accordance with the manufacturer's instructions. A DNase treatment was performed before RNA was eluted from the filter, and samples were stored at –80°C until analyzed. Interleukin-1, IL-6, and TNFα mRNA expression in blood samples was determined by use of a real-time RT-PCR assay.

A hyperinsulinemic, euglycemic clamp procedure was performed 6 hours after administration of saline solution and 6 and 24 hours after administration of LPS to identify changes in insulin sensitivity. Blood samples collected immediately prior to each clamp procedure were allowed to clot at 4°C overnight. Serum was harvested and stored at –20°C until submitted for determination of serum insulin concentration with a commercially available radioimmunoassay kit, as described. Each sample was analyzed in duplicate. The intra-assay coefficient of variance was 8.9%, and the detection limit for the assay was 1.9 µU/mL.

A hyperinsulinemic, euglycemic clamp procedure adapted for use in horses was performed. Briefly, three 6-mL blood samples were collected at 10-minute intervals to determine baseline insulin and glucose concentrations with glucose concentrations determined with a handheld meter previously validated for use in horses. Each mare was then given a bolus of insulin (0.4 mU/kg), and an infusion of insulin (1.2 mU·kg⁻¹·min⁻¹) was administered for 120 minutes. Two minutes after the insulin infusion was begun, a 50% glucose solution was administered at an initial rate of 30 mL/h. Blood samples were collected every 10 minutes for the first 40 minutes and then every 5 minutes thereafter, and blood glucose concentration was measured. The rate of glucose infusion was adjusted to maintain euglycemia, determined on the basis of baseline blood glucose concentration, for the entire 120-minute insulin infusion.

For determination of IL-1, IL-6, and TNFα mRNA expression in blood samples, RNA (500 ng) was diluted in 39 µL of nuclease-free water and combined with 41 µL of master mix (3 µL avian myeloblastosis virus RT* [20 U/µL]), 4 µL oligo dT primer* [0.5 µg/µL], 2 µL RNAsin® [40 U/µL], 8 µL dNTPmix® [10 mM], 8 µL avian myeloblastosis virus buffer, and 16 µL MgCl₂ (25 mM). Reaction mixtures were incubated at 42°C for 15
minutes, 95°C for 5 minutes, and 3°C for 5 minutes in a thermocycler. Samples of cDNA were then stored at −20°C until further analyzed.

Cytokine gene expression was measured in cDNA samples with a commercial system incorporating equine-specific TNFβ, IL-6, IL-1, and β-GUS primer and probe sets designed for this purpose. Expression of the housekeeping gene β-GUS did not vary more than 2-fold from the mean value. Primers and probes were designed to be intron spanning by comparison of the equine mRNA sequence with homologous splice sites reported for human sequences. For each primer-probe combination, assays with genomic DNA and with samples negative for RNA were performed to ensure that DNA was not amplified. For the RT-PCR assay, reaction mixtures were incubated at 95°C for 1 minute, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. Each reaction contained 20 µL of master mix (12.5 µL of a universal master mix²), 1.25 µL of 20X assay mix for the gene of interest (primer-probe set), 6.25 µL of nuclease-free water, and 5 µL of the cDNA template. All reactions were performed in duplicate wells.

Changes in cytokine gene expression were calculated by use of the ∆∆CT method, where ∆∆CT = {([gene of interest Ct − β-GUS Ct]sample − [gene of interest Ct − β-GUS Ct]calibrator), and Ct is defined as the amplification cycle at which the gene reaches a threshold level of fluorescence. Fold changes in gene expression were calculated as 2−∆∆CT. Results were expressed as the mean fold change in gene expression at each time point. The Ct for the control sample collected prior to administration of saline solution or LPS was used as the calibrator and assigned a fold change in expression of 1.

Experiment 2—Ten mares were used to determine changes in inflammatory cytokine mRNA expression in adipose tissue in response to LPS administration. Mares ranged from 10 to 16 years old; body condition score ranged from 4 to 6.

Mares were randomly assigned to treatment and control groups with 5 mares/group. Mares were housed in stalls with ad libitum access to water and hay throughout the experiment. An indwelling catheter was inserted into a jugular vein in each mare, and the next day, saline solution or LPS (0.045 µg/kg) was administered IV. Blood samples were collected hourly for 7 hours as described for experiment 1, and adipose tissue biopsy specimens were collected 2 and 8 hours after administration of saline solution or LPS. For collection of biopsy specimens, mares were sedated with xylazine hydrochloride (0.6 mg/kg, IV), acepromazine (0.04 mg/kg, IV), and butorphanol tartrate (0.02 mg/kg, IV). Adipose tissue specimens (100 mg) were collected from the fat pad near the tail head region, along with a core biopsy specimen (25 mg). Biopsy specimens were immediately flash frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated with a commercially available kit used in accordance with the manufacturer's instructions, with 2 exceptions. First, following disruption of the tissue with a handheld rotor-stator homogenizer and cell lysis with a reagent, an additional 10-minute centrifugation step was performed at 4°C. Second, 500 µL of chloroform was added to the lysate because this yielded greater RNA concentrations in preliminary tests. The protocol included a DNase I digestion step to ensure removal of any contaminating DNA. Total RNA was isolated from blood samples, and a real-time RT-PCR assay was used to measure inflammatory cytokine mRNA expression in adipose tissue and blood, as described for experiment 1.

Experiment 3—To determine whether equine adipocytes, in addition to infiltrated macrophages, produce inflammatory cytokines as part of the innate immune response in adipose tissue, subcutaneous adipose tissue specimens (100 g) were collected from the rumps of 2 aged (>25 years old) mares at necropsy. One mare had been euthanized because of blindness and the other because of severe arthritis and lameness.

Mature adipocytes were isolated as described, with the exception that washes were performed with phosphate-buffered saline solution containing 1% bovine serum albumin (pH, 7.4) and that samples were centrifuged at 500 X g for 5 minutes after each wash to remove stromal vascular and mononuclear cells. Alterations to the protocol were made on the basis of a previous report, which suggested that addition of bovine serum albumin protects cells against lysis and advocated the use of multiple centrifugations after each wash step to remove nonadipocyte cells. Mature adipocytes were used immediately to determine the short-term effects of LPS stimulation or put into ceiling culture to dedifferentiate into PAs, as described. Preadipocytes were grown to confluence in 25-cm² culture flasks and incubated in 10 mL of total medium with gentle shaking. Mature adipocytes (2 mL of packed floating cells) were incubated in 40 mL of total medium to allow enough surface area for a single layer of floating cells. Two replicates were used for each experimental condition.

Mature adipocytes and PAs were incubated separately for 1 or 3 hours in serum-free medium with or without the addition of LPS (10 µg/mL). Total RNA was then isolated with a commercially available kit used in accordance with the manufacturer's instructions, and RNA was stored at −70°C until analyzed. A real-time RT-PCR assay was subsequently used to quantify expression of IL-1, IL-6, and TNFβ, as described for experiment 1.

Additional samples of MAs and PAs were incubated for 4 hours in serum-free medium containing LPS (10 µg/mL) to which brefeldin A (1 µg/mL) had been added to block protein export from the Golgi apparatus. An intracellular staining protocol was then performed as described. Briefly, supernatant was removed, and cells were washed and fixed with 4% paraformaldehyde in 0.15M phosphate-buffered saline solution (pH, 7.4) for 20 minutes with gentle shaking at room temperature. Cells were then washed twice before being treated with 200 µL of phosphate-buffered saline solution containing 1% saponin, 3% fetal calf serum, and 1% bovine serum albumin for 45 minutes at room temperature. Cells were then incubated with murine monoclonal IgG, against equine TNFβ (HL801) or a negative isotype control (1 µg/mL) diluted in the same solution for 2 hours at room temperature with gentle shaking. Cells were washed 2 final times before they were resuspend-
ed in flow sheath fluid and analyzed by means of flow cytometric analysis.

For flow cytometric analysis, MAs and PAs were identified and gated on the basis of forward-scatter and side-scatter parameters; 10,000 events were acquired with a flow cytometer. Data were analyzed as total populations of MAs and PAs and as small cell and large cell fractions with standard software. Markers were set for samples incubated with the isotype control such that 1% of the cells were positive for TNF-α to account for background (nonspecific) fluorescence. These markers were then used to determine percentages of TNF-α-producing cells in LPS-stimulated MAs and PAs labeled with anti-TNF-α antibody. Results are expressed as the percentages of MAs and PAs that produced TNF-α in response to LPS stimulation, after subtraction of the isotype control value (1% by definition).

Statistical analysis—Mean glucose infusion rates for the clamp procedure were analyzed by means of general linear model analysis with the Dunnett 2-tailed t test for multiple comparisons. Temperature, pulse rate, and respiratory rate data for experiments 1 and 2 were analyzed by means of general linear model analysis. The fixed effects for these analyses were treatment, time, and the treatment X time interaction; the random effect was individual mares. Insulin concentrations 6 hours (day 4) and 24 hours (day 5) after LPS administration were compared with the baseline concentration (day 1) by means of general linear model analysis followed by the Student paired t test. Data for mRNA cytokine expression in blood were analyzed by means of general linear model analysis with the Tukey Studentized range test for multiple pairwise comparisons. Data for adipose tissue cytokine expression were not normally distributed and were, therefore, analyzed by means of the Mann-Whitney rank sum test.

Results

Experiment 1—Temperature, pulse rate, and respiratory rate (Figure 1) were significantly (P < 0.001) increased after LPS administration, compared with values obtained after administration of saline solution, with a

![Figure 1](https://example.com/figure1.png)

Figure 1—Mean body temperature, pulse rate, and respiratory rate after administration of saline solution (black circles) or LPS (white circles) in 5 mares. *Significantly (P < 0.05) different from value obtained at the same time point following administration of saline solution. †Significantly (P < 0.05) different from baseline (time 0) value for that group. Error bars represent SEM.

Table 1—Mean ± SD blood glucose and serum insulin concentrations in 5 mares immediately before (baseline) and during a hyperinsulinemic, euglycemic clamp procedure performed 6 and 24 hours after administration of LPS and 6 hours after administration of saline solution.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saline solution</th>
<th>6 hours</th>
<th>24 hours</th>
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</thead>
<tbody>
<tr>
<td>Blood glucose (mmol/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.65 ± 0.54a</td>
<td>9.91 ± 1.10b</td>
<td>6.53 ± 0.63a</td>
</tr>
<tr>
<td>During clamp procedure</td>
<td>4.65 ± 0.56c</td>
<td>9.75 ± 0.71b</td>
<td>6.89 ± 0.56c</td>
</tr>
<tr>
<td>Serum insulin (µU/mL)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.60 ± 2.24d</td>
<td>15.83 ± 8.34d</td>
<td>20.06 ± 7.35d</td>
</tr>
<tr>
<td>During clamp procedure</td>
<td>27.95 ± 3.26e</td>
<td>53.39 ± 6.82f</td>
<td>80.91 ± 17.24f</td>
</tr>
</tbody>
</table>

*Within treatment, there was a significant difference (P < 0.05) between baseline value and value obtained during the clamp procedure.

†Data for glucose values with different superscript letters were significantly different (P < 0.05) between treatments (saline solution and 6 and 24 hours after LPS administration).

Serum insulin values with different superscript letters were significantly different (P < 0.05) between treatments.
significant (P < 0.05) time effect and treatment X time interaction. Serum insulin concentration 6 hours (P < 0.05) and 24 hours (P < 0.001) after LPS administration was significantly increased, compared with concentrations measured after administration of saline solution (Table 1). Mares had a significant (P < 0.001) transient increase in insulin sensitivity 6 hours after LPS administration, compared with insulin sensitivity following administration of saline solution, followed by a significant (P < 0.001) decrease in insulin sensitivity 24 hours after LPS administration (Figure 2). There was also a significant effect of time (P < 0.001) and a significant treatment X time interaction (P < 0.001). Additionally, mares had significant (P < 0.001) increases in IL-1, IL-6, and TNFα expression in blood after LPS administration, compared with values obtained after administration of saline solution (Figure 3). There was a significant effect of time (P < 0.001) and a significant treatment X time interaction (P < 0.001). There was no significant change in cytokine expression over time when mares were given saline solution alone. In contrast, IL-1, IL-6, and TNFα expression was significantly (P < 0.05) increased 2 and 3 hours after LPS administration, compared with baseline expression.

Experiment 2—Mares had expected significant (P < 0.001) increases in temperature, pulse rate, and respiratory rate following administration of LPS, compared with values obtained following administration of saline solution (Figure 4). There were significant (P < 0.05) time effects for temperature, pulse rate, and respiratory rate and significant (P < 0.01) treatment X time interactions for temperature and respiratory rate, but not for pulse rate. Blood IL-1, IL-6, and TNFα mRNA expression were significantly (P < 0.001) increased following administration of LPS, compared with values obtained following administration of saline solution (Figure 5). There was a significant (P < 0.001) time effect and significant (P < 0.001) treatment X time interaction. In contrast with values obtained in mares treated with LPS, there was no change in cytokine expression over time in control mares. Adipose tissue mRNA expression of IL-1, IL-6, and TNFα was significantly (P < 0.05) increased 2 hours after administration of LPS, compared with values obtained following administration of saline solution (Figure 6). Expression of IL-1 and IL-6 in adipose tissue remained significantly (P < 0.01) increased 8 hours after LPS administration, compared with values obtained in control mares. However, TNFα expression was not significantly different between groups at 8 hours.

Experiment 3— Cultured PAs stained positive for TNFα after incubation with LPS, with 60.18% of the cell population positive for TNFα (Figure 7). Isolated MAs also stained positive for TNFα, although to a lesser degree than PAs; 14.68% of the total population of MAs produced TNFα. Expression of IL-1, IL-6, and TNFα was not detected in unstimulated MAs, and only IL-6 expression was detected in unstimulated PAs. Therefore, unstimulated MAs could not be used as a calibrator for analysis of LPS-stimulated MAs. Likewise, unstimulated PAs could not be used as a calibrator for analysis of IL-1 and TNFα expression in LPS-stimulated PAs. Thus, unstimulated PAs were used as a calibrator (ie, fold change in expression was designated 1) for IL-6 expression in PAs and MAs, whereas PAs that were incubated for 3 hours with LPS were used as a calibrator (ie, fold change in expression was designated 1) for analysis of IL-1 and TNFα expression. Analysis of real-time RT-PCR assay results revealed that both PAs and MAs expressed IL-6 and TNFα in response to LPS stimulation (Figure 8). In addition, IL-1 was expressed in stimulated PAs, but was not detected in MAs. Relative mRNA expression was greater for PAs and MAs incubated with LPS for 1 hour than for cells incubated with LPS for 3 hours.

Discussion

The results of experiment 1 in the present study indicated for the first time that an inflammatory response stimulated by LPS administration induces insulin resistance within 24 hours in horses. In other species, the innate immune response to endotoxin or sepsis is biphasic, characterized by initial hyperglycemia and increased metabolic glucose clearance followed by hypoglycemia and insulin resistance.7-9,28-30 Similarly, in the present study, insulin sensitivity was increased 6 hours after LPS administration, compared with insulin sensitivity after administration of saline solution. This same response is seen within 2 hours of LPS administration in humans.30 The prolonged increase in insulin sensitivity after LPS administration in horses, compared with other species, may be due to their relatively larger body size, requiring more time to elicit whole-body metabolic changes. The initial increase in insulin sensitivity corresponded with a 4-fold increase in circulating insulin.
concentration, compared with baseline concentration, indicating an upregulation of glucose disposal as part of the initial inflammatory response.

The initial increase in insulin sensitivity seen in the present study could also have been the result of cytokine-stimulated non–insulin-mediated glucose uptake in macrophage-rich tissues. In humans, hyperglycemia initially develops following IL-1β and TNFα-mediated stimulation of catecholamine and epinephrine release, which in turn increase hepatic glucose output. Glucose utilization is also increased, mainly via non–insulin-stimulated glucose uptake, which results from enhanced glucose utilization by tissues rich in mononuclear phagocytes, including the liver, spleen, lung, and ileum. Infusion of TNFα increases non–insulin-stimulated glucose uptake in vivo and may act via stimulation of catecholamines, glucagon, or corticosterone. It also stimulates glucose uptake by macrophages by upregulating the non–insulin-dependent glucose transporter GLUT-1.

When the clamp procedure was repeated 24 hours after LPS administration, a marked decrease in insulin sensitivity, compared with sensitivity following administration of saline solution, was observed, despite an increase in blood insulin concentration. Although the exact mechanisms for LPS-induced insulin resistance have yet to be elucidated, the results of the present study suggest that the innate immune response affects insulin sensitivity in horses.

Results of experiment 2 in the present study indicated that adipose tissue in horses responds to an inflammatory challenge with a significant upregulation in mRNA expression of the inflammatory cytokines IL-1, IL-6, and TNFα. There was a slightly decreased physiologic response (pulse and respiratory rate) 2 to 3 hours after administration of LPS or saline solution in experiment 2 versus experiment 1. This was likely the result of drug administration immediately prior to adipose tissue biopsy, as decreases were observed in both groups and values increased again within hours.

![Graphs showing cytokine expression and temperature, pulse, and respiratory response after LPS or saline administration.](image)

Figure 3—Relative expression (ie, fold change in gene expression relative to a calibrator) of IL-1, IL-6, and TNFα mRNA in blood after administration of saline solution (black circles) or LPS (white circles) to 5 mares. See Figure 1 for key.

Figure 4—Mean body temperature, pulse rate, and respiratory rate after administration of saline solution (black circles) or LPS (white circles) to mares (n = 5/group). See Figure 1 for key.
Decreases in pulse and respiratory rates in response to anesthetics and analgesics used in the present study have been reported previously. Nevertheless, despite the temporarily reduced physiologic response to LPS, there was a significant upregulation in adipose tissue cytokine expression. These results provide the first evidence of the potential contribution of adipose tissue to the inflammatory response in horses.

The purpose of experiment 3 in the present study was to obtain a qualitative, rather than quantitative, assessment of the inflammatory cytokine response in MAs and PAs in horses in an attempt to determine whether these adipose tissue constituents participate in the innate immune response. Intracellular staining of LPS-stimulated MAs and PAs revealed that both groups of adipocytes produce TNFα. However, a greater percentage of PAs produced TNFα than MAs. This finding is supported by results of a recent study in which most inflammatory cytokines produced in humans in response to an inflammatory challenge were found to originate from PAs rather than MAs. Stimulation of equine adipocytes with LPS also resulted in upregulation of mRNA expression of IL-6 and TNFα; IL-1 mRNA expression was observed to a small degree in PAs, but not in MAs. Although IL-1 expression in adipose tissue results mainly from infiltrative macrophages, an in vitro study involving 3T3-L1 adipocytes demonstrated that PAs have many characteristics of macrophages, and PAs are known to produce IL-1. These results provide initial qualitative evidence that adipocytes participate in the innate inflammatory response in adipose tissue in horses, although further studies are needed to quantify the nature of their contribution.

We have recently demonstrated an association between obesity, insulin resistance, and increased concentrations of inflammatory cytokines in horses, and long-term exposure to the inflammatory cytokines IL-1, IL-6, and TNFα has been shown to induce insulin re-

Figure 5—Relative expression (ie, fold change in gene expression relative to a calibrator) of IL-1, IL-6, and TNFα mRNA in blood after administration of saline solution (black circles) or LPS (white circles) to mares (n = 5/group). See Figure 1 for key.

Figure 6—Relative expression (ie, fold change in gene expression relative to a calibrator) of IL-1, IL-6, and TNFα mRNA in adipose tissue 2 and 8 hours after administration of saline solution or LPS in mares (n = 5/group). See Figure 1 for key.
stance in other species.\textsuperscript{32,33,39} In obese humans and rodents, there is evidence that high circulating concentrations result in part from overproduction of adipose-tissue-derived inflammatory cytokines,\textsuperscript{13} and results of the present study suggested that equine adipose tissue also has the ability to produce cytokines. Therefore, it is possible that inflammation observed in obese horses in our previous study\textsuperscript{13} may relate to increased production of inflammatory cytokines by adipose tissue. Furthermore, results of the present study suggest that PAs and MAs can contribute to inflammatory cytokine production in equine adipose tissue. In addition, research in other species suggests that adipocyte deposits associated with obesity induce increased macrophage recruitment, activation, and infiltration into adipose tissue.\textsuperscript{40} Cross-talk between adipocytes and macrophages creates a vicious cycle of inflammation in obese individuals.\textsuperscript{51}

Therefore, the accumulation of excess adipose tissue may contribute to high inflammatory cytokine production in obese horses.

Results of experiment 1 in the present study may have important clinical implications in the field of equine medicine. Injury and critical illness in humans are characterized by hyperglycemia, high free fatty acids concentrations, and protein catabolism, in part because of suppression of insulin secretion during the “shock phase” and insulin resistance during the “flow phase” of injury, accompanied by high concentrations of cytokines, cortisol, glucagon, and catecholamines.\textsuperscript{42} In humans, there is considerable evidence that insulin resistance develops immediately after even minor elective surgery in conjunction with increases in circulating concentrations of inflammatory cytokines.\textsuperscript{43–51} The degree and duration of insulin resistance depend on the magnitude of the operation performed.\textsuperscript{46} In addition, sepsis is associated with increases in circulating concentrations of the inflammatory cytokines IL-1, IL-6, and TNF\textsubscript{a}, as well as hyperglycemia.\textsuperscript{47} Insulin therapy suppresses production of cytokines and free radicals, enhances production of the anti-inflammatory cytokines interleukin-4 and interleukin-10, corrects stress hyperglycemia, and improves myocardial function during sepsis.\textsuperscript{47} In addition, preoperative or perioperative insulin or glucose therapy and preoperative carbohydrate loading attenuate postoperative insulin resistance and systemic inflammation.\textsuperscript{49–53} There is also evidence to suggest that glycemic control during surgery improves clinical outcome in individual patients.\textsuperscript{54,55} Therefore, insulin or glucose therapy may have practical applications in equine medicine and surgery.

Results of the present study may also have relevance to laminitis, which can occur when inflammation develops, such as after surgery or trauma,\textsuperscript{54,55} and is often associated with obesity.\textsuperscript{56} Impaired glucose uptake associated with inflammation in obesity and postoperative conditions may weaken layers of the hoof lamellae and predispose horses to laminitis when other pathways are activated.\textsuperscript{57,58} Therefore, future studies are needed to examine the efficacy of insulin-sensitizing agents and preoperative insulin or glucose therapy in combination with anti-inflammatory medications in protecting against the development of laminitis in horses.

Figure 7—Log values for relative fluorescence intensity (x-axis) versus relative number (y-axis) of PAs after incubation with LPS and intracellular staining for fluorescein isothiocyanate (FITC)-labeled TNF\textsubscript{a} (black area) or a FITC-labeled isotype control (gray line). Fluorescence covered by the area of the isotype control antibody represents nonspecific staining. The area underneath the horizontal bar represents TNF\textsubscript{a}-specific fluorescence of PA.

Figure 8—Relative expression of IL-1, IL-6, and TNF\textsubscript{a} mRNA in MAs and PAs that were incubated with LPS for 1 (1H) or 3 (3H) hours and for unstimulated control MAs and PAs. See Figure 1 for key.
References


