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Effect of Lipopolysaccharide on Indices of Peripheral and Hepatic Metabolism in Lactating Cows[1]

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ABSTRACT

Four multiparous lactating cows (175 to 220 d in milk [DIM]) were used in a 4 × 4 Latin square design to assess the effects of four doses (0.0, 0.5, 1.0, and 1.5 μg/kg of body weight) of lipopolysaccharide (LPS; *Escherichia coli* 0111:B4) on performance and plasma metabolite and hormone concentrations. In addition, effects of immune activation on in vitro hepatic metabolic capacity were evaluated in 12 multiparous lactating cows (150 to 220 DIM) infused with 0 (n = 6), 1.0 (n = 4) or 2.0 (n = 2) μg of LPS/kg. Milk production and DMI decreased linearly with LPS dose for 24 h after LPS infusion. Overall mean plasma tumor necrosis factor-α, insulin, glucagon, and cortisol concentrations increased linearly with LPS dose, and plasma β-hydroxybutyrate decreased linearly by dose after LPS infusion. Infusion of LPS decreased the insulin:glucagon molar ratio, but did not affect plasma concentrations of growth hormone, insulin-like growth factor-1, leptin, or L-(+)-lactate. Plasma concentrations of glucose tended to increase initially and subsequently decrease, and there was a quadratic tendency for increased plasma nonesterified fatty acid concentrations after LPS administration. In vitro hepatic capacity for conversion of [1-^{14}C]L-(+)-lactate and [1-^{14}C]palmitate, but not [1-^{14}C]propionate or [1-^{14}C]L-alanine, to CO_2 increased after LPS administration. Hepatic capacity to convert [1-^{14}C]propionate to glucose tended to increase, but neither esterification nor the conversion of palmitate to acid soluble products was altered by LPS. The LPS infusion resulted in significant changes of endocrine mediators responsible for regulation of energy metabolism of lactating cows and tended to alter subsequent in vitro hepatic metabolic capacity.

(**Key words:** lipopolysaccharide, hormone, metabolism, liver)

Abbreviation key: GH = growth hormone, **LPS** = lipopolysaccharide, **PBST** = PBS with Tween 80 (0.1% vol/vol), **PBST-g** = PBS with Tween 80 (0.1% vol/vol) and gelatin (0.5% vol/vol), **PIH** = post-infusion hour, **rBoTNF-α** = recombinant bovine TNF-α, **RIA** = radioimmunoassay, **TNF-α** = tumor necrosis factor-α.

INTRODUCTION

Gram-negative bacteria are responsible for the pathology of many diseases in livestock production. In dairy cattle, neonatal septicemia, mastitis, certain diarrheas and pneumonias, and metritis are among the infections commonly caused by these organisms (Cullor, 1992). Because infectious disease can negatively impact productive processes (Johnson, 1997; Spurlock, 1997), it is important to minimize its occurrence in order to maintain profitability in livestock production systems. Rajala-Schultz et al. (1999) reported that in dairy cattle, mastitis had a sustained effect on milk yield resulting in 110 to 552 kg of lost milk per lactation and cows with mastitis failed to reach their premastitis milk yields during the remainder of the lactation after the onset of infection. Furthermore, mastitis is an important risk factor for culling (Grohn et al., 1998). For these reasons, the immunologic consequences of infectious disease in dairy cattle have been studied extensively.

There is a dearth of information regarding the effects of infectious disease on the metabolism of lactating dairy cattle. The study of the metabolic consequences of immune activation in dairy cattle has emphasized mechanisms of decreased milk production (Shuster and Harmon, 1992; Shuster et al., 1995), effects within the mammary gland (Shuster et al., 1993; Shuster and Kehrli, 1995), and to a lesser extent, effects on reproduction (Leung et al., 2001; Suzuki et al., 2001). Other research has focused on the potential for specific nutrients or metabolites to impact immune function (Burton et al., 1993; Franklin et al., 1995; Ametaj et al., 2000). Although Curtis et al. (1985) reported associations between infectious and metabolic disease, information de-

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scribing the effect of immune activation on metabolism in lactating dairy cows is limited.

The inflammatory response to gram-negative bacterial invasion is caused by the host reaction to endotoxin, the lipopolysaccharide (**LPS**) cell wall component of gram-negative bacteria (Luderitz et al., 1984). Purified LPS administered to animals mimics the clinical symptoms caused by bacterial infection (Memon et al., 1992; Steiger et al., 1999). Symptoms include lethargy, respiratory distress, fever, hypotension, and tachycardia, ultimately causing decreased cardiac output, diarrhea, changes in blood cell counts, and alterations in the blood coagulation system (Cullor, 1992). Metabolic disturbances also accompany clinical manifestations of endotoxemia. Anorexia, as well as alterations in carbohydrate, fat, protein, and mineral metabolism are all components of the pathological response elicited by LPS (Klasing, 1988). The metabolic response to bacterial sepsis or LPS administration is mediated primarily via cytokine and endocrine control, and there is little effect of endotoxin directly on tissues (Filkins and Buchanan, 1977).

The objective of this study was to characterize the effect of immune activation induced by LPS administration on multiple aspects of intermediary metabolism in lactating dairy cows. We hypothesized that immune activation would result in the perturbation of regulatory endocrine factors that would result in alteration of metabolite concentrations in the peripheral circulation and hepatic metabolic capacity.

MATERIALS AND METHODS

Longitudinal Metabolism Experiment

Experimental design. All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee. Four multiparous lactating Holstein cows (175 to 220 DIM) were used in a 4 × 4 Latin square design (balanced for carryover effects) with 7-d periods to assess the effects of four doses (0.0, 0.5, 1.0, and 1.5 μg/kg of BW) of bacterial LPS (*Escherichia coli* 0111:B4, Sigma Chemical Co., St. Louis, Mo.) on feed intake, milk production, and blood chemistry. Cows at the Cornell University Teaching and Research Dairy Farm were housed in tie stalls, moved to metabolism stalls for the infusion period, and milked three times per day throughout the experiment.

Infusions. The infusion protocol used was modeled after that of Werling et al. (1996) and Steiger et al. (1999), except that LPS-infused animals were not pair-fed with controls. Bilateral jugular vein catheters were fitted 24 h before infusion and were removed 24 h post infusion (**PIH**). Treatments were dissolved in 100 ml of 0.9% NaCl sterile saline and infused intravenously through a jugular catheter over a period of 100 min. Infusions were administered intravenously via a Plum XL infusion pump (Abbott Laboratories, North Chicago, IL) with a 0.2-μM low-protein binding in-line filter (Gelman Sciences, Ann Arbor, MI). The LPS dose (i.e., 0.0, 0.5, 1.0, 1.5 μg/kg of BW) for a given infusion day was calculated based on average BW for the 3 d before infusion. All cows were infused on d 7 of each period. Infusions began about 30 min after the second daily milking (~0930 h), and on experimental days, cows were offered their daily ration at the initiation of infusion. Intakes for individual cows were measured at the end of the 8-h sampling period and daily throughout the experimental period.

Sampling. Milk weights were recorded at each milking during the experimental period. Blood was sampled just before infusion (0 h), at hourly intervals thereafter through 8 h, and again at 24 and 48 h. During the initial 24 h after LPS infusion, blood was taken via the contralateral catheter. The 48-h blood sample was taken via the coccygeal vein into heparinized vacuum sampling tubes (20 U/ml blood, Becton Dickinson, Franklin Lakes, NJ). Twenty milliliters of blood was sampled at each sampling timepoint. Blood samples were placed on ice and centrifuged (2060 × g, 15 min, 5°C). Harvested plasma was immediately frozen and stored at –20°C for subsequent analysis. Kallikrein protease inhibitors, aprotinin and leupeptin (500 KIU/ml and 25 μg/ml plasma, respectively; Sigma Chemical Co.), were added to plasma aliquots for glucagon analysis before freezing.

Clinical measurements. All measurements were made immediately before LPS infusion (0 h), at 30-min intervals during the initial 8 PIH, and again at 24 and 48 h. Rectal temperature was measured using a digital thermometer, heart rate was measured by auscultation using a stethoscope placed on the thoracic cavity, and respiration rate was recorded following timed observation of the abdominal wall. Internal body temperature was recorded using a radiotelemetric temperature recording system (CowTemp, Innotek, Inc., Garrett, IN) and transmitter located in the reticulo-rumen. These data are not included due to unreliability of the data. For example, reticuloruminal temperature typically would decrease by 5°C (or more) after drinking. These short-duration (30 to 60 min) effects would not likely affect hourly or daily trends for health or reproductive monitoring, but introduced variation that made the data unusable.

Analyses. Metabolite assays were conducted in 96-well microplates (Costar, Corning Inc., Acton, MA) and read using a microplate reader spectrophotometer (Molecular Devices, Sunnyvale, CA). Plasma metabolites were analyzed by enzymatic colorimetric assays using

procedures modified from available kits (glucose [kit 510A], L-(+)-lactate [kit 735-10], and BHBA [kit 310-UV], Sigma-Aldrich Co.; NEFA, Wako Chemicals USA Inc., Dallas, TX) and validated in our laboratory.

Plasma hormone concentrations were analyzed by radioimmunoassay (**RIA**) procedures modified from published assay procedures (leptin, growth hormone [**GH**], IGF-1) or commercially available kits (insulin and glucagon from Linco Research, Inc., St. Louis, MO; cortisol from Diagnostic Systems Laboratories, Inc., Webster, TX) validated in our laboratory. Plasma leptin was measured using the procedure of Ehrhardt et al. (2000) and bovine insulin used for iodination and standards for the insulin RIA were provided as a gift from L. Richardson (Elanco Animal Health, Greenfield, IN). Intra- and interassay coefficients of variation were 9.0 and 10.2%, 4.1 and 14.1%, 9.4 and 6.9%, and 4.2 and 14.1% for the insulin, glucagon, cortisol, and leptin RIA, respectively.

Plasma GH was determined by double-antibody RIA as described by Cohick et al. (1986). Recombinant bovine GH (lot 12, code 77-001; Pharmacia & Upjohn Inc., Kalamazoo, MI) was used for iodination and standards. Primary antibody was supplied from NIDDK (A. F. Parlow, National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) and secondary antibody was a gift from W. R. Butler (Department of Animal Science, Cornell University). Intra- and interassay coefficients of variation were 5.7 and 5.6%, respectively.

Plasma concentrations of IGF-1 were assayed by a double-antibody RIA procedure after acid-ethanol extraction as described by Rosemberg et al. (1989). Rabbit anti-human IGF-1 (AFP4892898, NIDDK) was used at a working concentration of 1:450,000, and purified IGF-1 (lot GTS-2, Monsanto Co., St. Louis, MO) was radioiodinated with iodogen as the oxidizing agent. Secondary antibody was a gift from W. R. Butler. Intra- and interassay coefficients of variation for the IGF-1 RIA were 4.0 and 8.6%, respectively.

Plasma concentrations of tumor necrosis factor-α (**TNF-α**) were measured by capture-ELISA (reagents provided by Veterinary Infectious Diseases Organization, Saskatoon, Canada). Assays were performed in Immunolon II microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). Reagents consisted of a capture antibody (mouse ascites anti-TNF-α, IgG fraction), detection antibody (rabbit anti-bovine-TNF-α, IgG fraction), recombinant bovine TNF-α (**rBoTNF-α**), biotinylated goat anti-rabbit IgG (Zymed Laboratories, Inc., South San Francisco, CA), horseradish peroxidase-conjugated streptavidin-biotinylated complex (Amersham Corp., Arlington Heights, IL), substrate (H202 at 0.1 % vol/vol) and dye (2,2'-azinodi-ethylbenzothiazoline-sulphonic acid). Internal standards of serially diluted rBoTNF-α were prepared in PBS (pH 7.2, 0.01 *M*) with Tween 80 (0.1 % vol/vol, **PBST**) and gelatin (0.5 % vol/vol, **PBST-g**). Positive and negative controls and serum samples were diluted serially in PBST-g. Capture antibody was diluted (1:1000 vol/vol) in carbonate buffer (pH 9.6, 0.01 *M*), and detection antibody in PBST-g (1:1500 vol/vol). Biotinylated goat anti-rabbit Ig was diluted 1:10,000 and horseradish peroxidase-conjugated streptavidin-biotinylated complex 1:2000 in PBST-g. Intervening washes were done with PBST. Enzyme substrate and indicator dye were diluted in citrate buffer. All incubations were at room temperature with the exception of capture antibody in carbonate buffer, which was incubated at 4°C. Absorbances of standards and test samples were read at 405 and 490 nm using an ELISA plate-washer and reader (Dynatech MR7000). The concentration of TNF-α in test samples was determined by comparing the absorbency of test samples with the absorbency of standards within a linear curve fit. Intra- and interassay coefficients of variation were 6.8 and 12.4%, respectively.

Liver Metabolic Capacity Experiment

Experimental design. All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee. Twelve multiparous lactating Holstein cows (150 to 220 DIM) were used in a completely randomized design to assess the effects of three doses (0.0, [n = 6]; 1.0, [n = 4]; and 2.0, [n = 2] μg/kg of BW) of intravenous bacterial LPS (*Escherichia coli* 0111:B4, Sigma Chemical Co.) on subsequent in vitro liver slice metabolism. The 2.0 μg/kg of BW treatment group received only two replicates because there was an interaction between this dose of LPS administered and the liver biopsy procedure that proved fatal for cows assigned to that treatment. Subsequent investigation into the death of those cows was inconclusive, but the treatment was discontinued. Cows at the Cornell University Teaching and Research Dairy Farm were housed in tie stalls, moved to metabolism stalls for the infusion and liver biopsy sampling period, and milked 3× throughout the experiment.

Infusions. The LPS infusion protocols were as described above in the longitudinal metabolism section of this paper, except that saline-infused animals were pair-fed with LPS-infused animals during the infusion period to eliminate potential confounding effects of feed intake with LPS treatment on liver metabolism.

Sampling. Samples of liver (~4 g) were obtained via percutaneous trochar liver biopsy (Veenhuizen et al., 1991) 7 d before LPS or saline infusion (covariate) and 4.5 h after the onset of saline or LPS infusion. The liver

sample was blotted to remove blood and loose tissue. A portion of the liver was snap-frozen in liquid N and the remainder was immersed in ice-cold 0.02 *M* PBS (0.9 %; pH = 7.4) for transport to the laboratory. Liver samples were returned to the laboratory within 1 h of biopsy for metabolic incubations as described below.

Metabolic incubations. Liver slices, prepared using a mechanical tissue slicer (Krumdieck Tissue Slicer, Alabama Research and Development, Munford, AL), were used for in vitro incubations to determine hepatic capacities for conversion of [1-^{14}C]propionate, [1-^{14}C]L-alanine, and [1-^{14}C](L+)-lactate to CO_2. Additionally, liver slice incubations were performed to determine hepatic capacity for conversion of [1-^{14}C]propionate to glucose following LPS or saline infusion. Unfortunately, procedural errors for the study of gluconeogenic capacity of covariate liver samples produced unusable data for this measurement during the covariate period. Oxidative and gluconeogenic capacity were measured using the methods of Overton et al. (1999) except that Krebs-Ringer bicarbonate media did not contain phenol red, incubation times were extended to 120 min, and recovery and measurement of newly synthesized glucose in the incubation media were conducted using the slurry ion exchange resin method of Azain et al. (1999).

Hepatic capacities for conversion of [1-^{14}C]palmitate to one of several metabolic products were determined on all liver samples. Conversion of radiolabeled palmitate to CO_2 or acid-soluble products (a proxy for ketone body production) was determined using methods of Drackley et al. (1991b), and conversion of radiolabeled palmitate to stored esterified products was determined using methods of Drackley et al. (1991a). The incubation medium was Krebs-Ringer bicarbonate buffer (pH 7.4); however, part of the NaCl (25 m*M*) was replaced by sodium HEPES (6.5 g/L) and 1.0 m*M* D, L-carnitine was added to all flasks.

Similar incubation procedures were used for the above analyses of hepatic metabolic capacity (Drackley et al., 1991a,b; Overton et al., 1999). Briefly, liver-slice (35 to 80 mg) incubations were conducted in water baths at 37°C in 25-ml Erlenmeyer flasks containing Krebs-Ringer bicarbonate buffer (pH 7.4). Radiolabeled propionate, L-alanine, and L(+)-lactate (0.9 μCi/flask) were included in the incubation media with unlabeled substrate at a final concentration of 10 m*M*, and radiolabeled palmitate (0.5 μCi/flask) was included in incubation with unlabeled substrate at a final concentration of 2 m*M*. Liver-slice incubations were terminated after either 0 or 120 min, and hepatic conversion of substrates was determined by recovery of radiolabeled products. Carbon dioxide was scavenged within incubation flasks using NaOH-soaked filter paper in hanging center-wells. Stored esterified products were recovered from liver slices extracted with hexane isopropanol (2:1), and all other measured metabolites were recovered from the incubation media. All radiolabeled conversion products were suspended in scintillation cocktail (Scintisafe Econo 2; Fisher Scientific, Pittsburgh, PA), and radioactivity was measured using liquid scintillation spectroscopy (model 2200 CA, Packard Instrument Co., Downers Grove, IL). All radiolabeled chemicals were from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

Statistical Analyses

Longitudinal metabolism experiment. Data were analyzed as a Latin Square design with repeated measures using the GLM procedure with the appropriate specified error term (SAS Inst., Inc., Cary, NC). Means were adjusted by analysis of covariance using data collected from each cow before LPS or control infusion and analysis of variance was conducted as described above. Cow was the experimental unit and classification variables were cow, period of the Latin square, treatment, and time of sampling. The model statement included cow, period, treatment, the interaction of cow, period and treatment, time, and the interaction of treatment and time. The overall effect of treatment was tested using the interaction of cow, period, and treatment as the error term. Linear, quadratic, and cubic effects of LPS treatment for each variable were tested using orthogonal contrasts using the interaction of cow, period, and treatment as the specified error term. Reported means are adjusted means ± the standard error of the mean. Significance was declared at $P < 0.05$ and trends from $P > 0.05$ to < 0.15.

Liver metabolic capacity experiment. Triplicate incubations were averaged and blanks (flasks containing tissue killed after 0 min) were subtracted from flasks containing tissue killed after 120 min of incubation before statistical analysis. Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc.). For data with covariate sampling, means were adjusted by analysis of covariance using data collected from each cow before LPS or control infusion, and ANOVA was conducted as described above. Cow was the experimental unit and cows were blocked according to date of infusion. Block and LPS treatment were classification variables and LPS treatment was included in the model statement. Treatment effects were tested using the residual error term. Linear and quadratic effects of LPS treatment for each variable were tested using orthogonal contrasts. Reported means are least squares means ± the standard error of the mean.

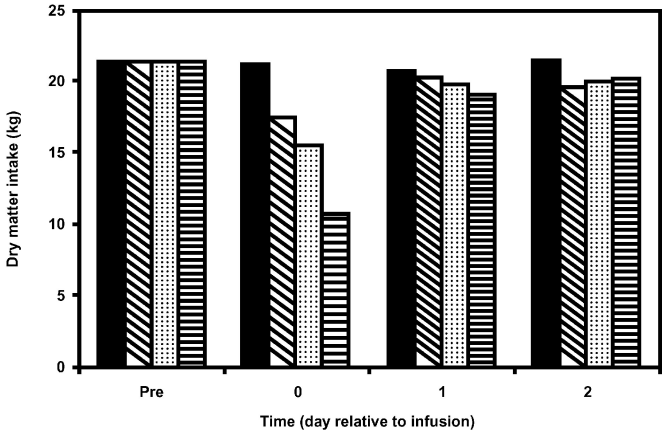

Figure 1. Daily DMI (kg; SE = 1.29)* following 0.0 (solid), 0.5 (diagonal), 1.0 (stippled), or 1.5 (horizontal) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows**. Means were adjusted by analysis of covariance. *"Pre" refers to the average daily DMI of d –2 through –4. **Treatment × time effect, P = 0.03; linear trend, P = 0.06.

RESULTS

Dry matter intake decreased linearly with LPS dose (P = 0.007) for the first 8 PIH and was 12.6, 9.0, 5.5, and 4.3 kg (SE = 1.58) for 0.0, 0.5, 1.0, and 1.5 μg of LPS/kg of BW. Similarly, daily DMI decreased following LPS infusion relative to the average daily DMI for 2 through 4 d before infusion (treatment × time, P = 0.03; linear trend, P = 0.06; Figure 1). The decrease in intake was transient since DMI for treated cows was not different from control cows other than on the day of infusion. Milk production per milking also decreased following LPS infusion (treatment × time, P = 0.001; linear trend, P = 0.07, Figure 2) and returned to preinfusion levels within 48 to 72 h after infusion.

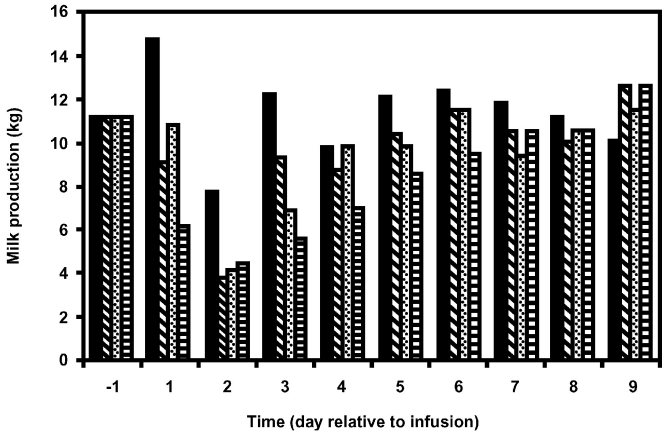

Figure 2. Milk production (kg; largest SE = 1.0) at each milking following 0.0 (solid), 0.5 (diagonal), 1.0 (stippled), or 1.5 (horizontal) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows*. Means were adjusted by analysis of covariance. *Treatment × time effect, P = 0.001; linear trend, P = 0.07

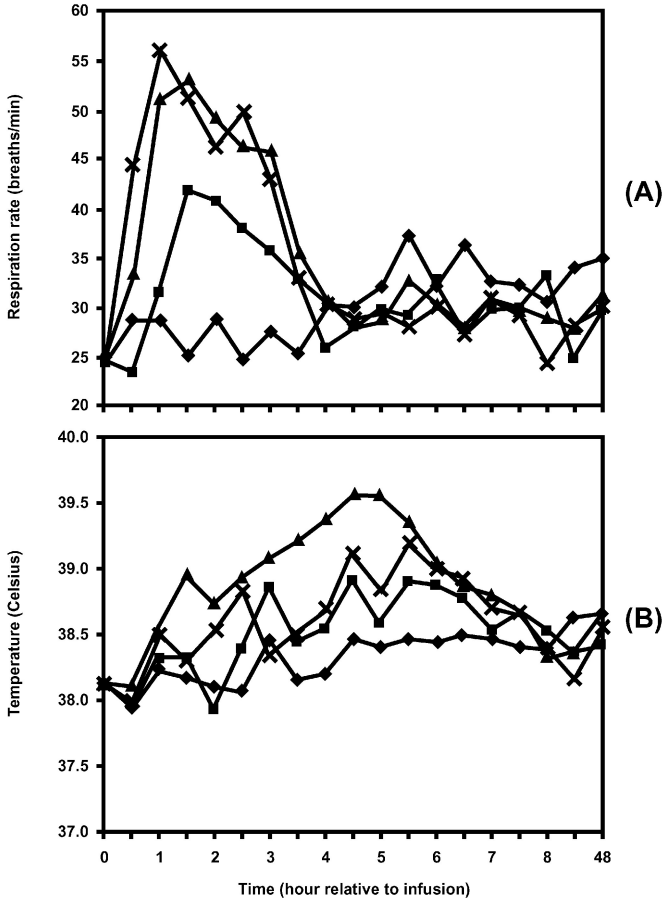

Figure 3. Respiration rate (Panel A; largest SE = 4.72)* and rectal temperature (Panel B; largest SE = 0.21)** following 0.0 (diamond), 0.5 (square), 1.0 (triangle), or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. *Treatment × time effect, P = 0.001; linear tendency, P = 0.12. ** Treatment × time effect, P = 0.02; quadratic effect, P = 0.02

Clinical measurements with significant responses are shown in Figure 3. Respiration rate increased sharply following LPS infusion, peaked by 1.5 PIH, and returned to preinfusion and control (0 LPS) infusion levels by 4 PIH (treatment × time, P = 0.001; linear tendency, P = 0.12). Rectal temperatures were less affected and peaked later than respiration rate, but were increased relative to saline-infused cows (treatment × time, P = 0.02; quadratic effect, P = 0.02). Heart rate postinfusion was highly variable and was not affected (P > 0.20) by LPS infusion (data not shown).

Concentrations of TNF-α in plasma following LPS infusion are shown in Figure 4. Effects of LPS on plasma TNF-α increases were dose-dependent (treatment ×

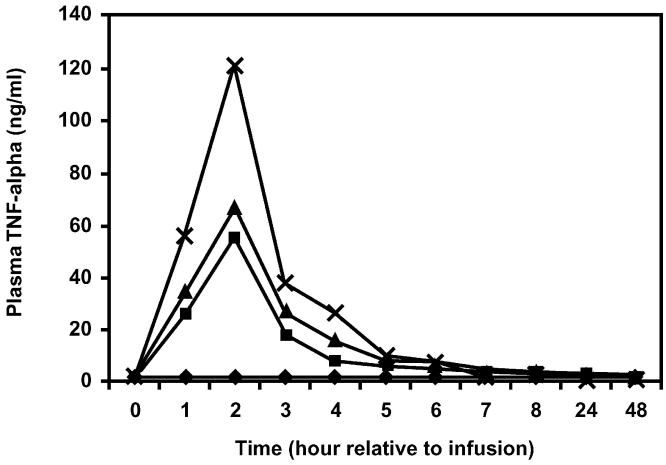

Figure 4. Plasma tumor necrosis factor-alpha concentration (TNF-α; SE = 9.7)* following 0.0 (diamond), 0.5 (square), 1.0 (triangle), or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. *Treatment × time effect, P = 0.001; linear effect, P = 0.05.

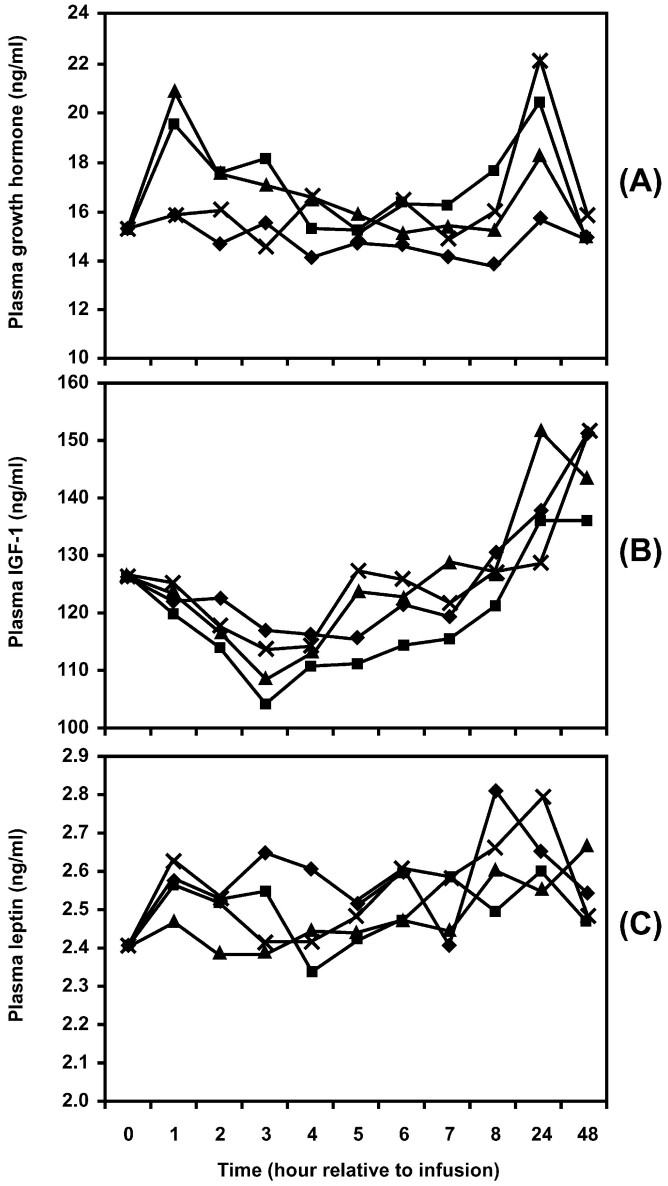

Figure 5. Plasma growth hormone (Panel A; SE = 1.57)*, insulin-like growth factor-1 (IGF-1; Panel B; SE = 7.3)*, and leptin (Panel C; SE = 0.09)* concentrations following 0.0 (diamond), 0.5 (square), 1.0 (triangle), or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. *No effect of lipopolysaccharide, P > 0.20.

time effect, P = 0.001; linear effect, P = 0.05). Of note, plasma TNF-α concentrations increased significantly by 1 PIH and returned to basal levels by 5 PIH.

Infusion of LPS had no effect on plasma GH, IGF-1, or leptin concentrations (Figure 5); however, plasma insulin (treatment × time, P = 0.007) and glucagon (treatment × time, P = 0.001; linear effect, P = 0.01) concentrations were significantly altered following infusion (Figure 6). Plasma insulin and glucagon concentrations peaked at 3 PIH, glucagon was elevated from 2 to 8 PIH, and insulin was lower than control for all timepoints measured other than 3 PIH. The molar ratio of plasma insulin to glucagon concentrations decreased (treatment × time, P = 0.01; linear effect, P = 0.001) from 1 to 8 h after infusion of LPS (Figure 6). Although effects of LPS infusion on glucoregulatory hormones were significant, differences in plasma glucose or L-(+)-lactate concentrations were not detected (Figure 7). Infusion of LPS caused an initial hyperglycemia followed by hypoglycemia, although this effect was not significant (P = 0.13).

Although LPS infusion had no effect on circulating energy metabolites, and despite the low number of replicates especially for the 2 μg LPS dose, in vitro metabolism of some glucogenic precursors by liver slices was altered following LPS infusion. Oxidation of ^{14}C-labeled glucogenic precursors to CO_2 is shown in Figure 8. L-(+)-Lactate (linear effect, P = 0.01), but not propionate (P > 0.20) or L-alanine (P > 0.20), oxidation to CO_2 increased following increasing doses of intravenous LPS. There also tended (P = 0.07) to be a positive association between the conversion of propionate to glucose and the amount of LPS administered (Figure 9).

Plasma cortisol concentration increased in a dose-dependent manner (treatment × time and linear effects, P = 0.001) following intravenous LPS infusion into midlactation cows (Figure 10), and there was a quadratic tendency (P = 0.13) for plasma NEFA concentration to increase. Plasma NEFA concentrations following LPS

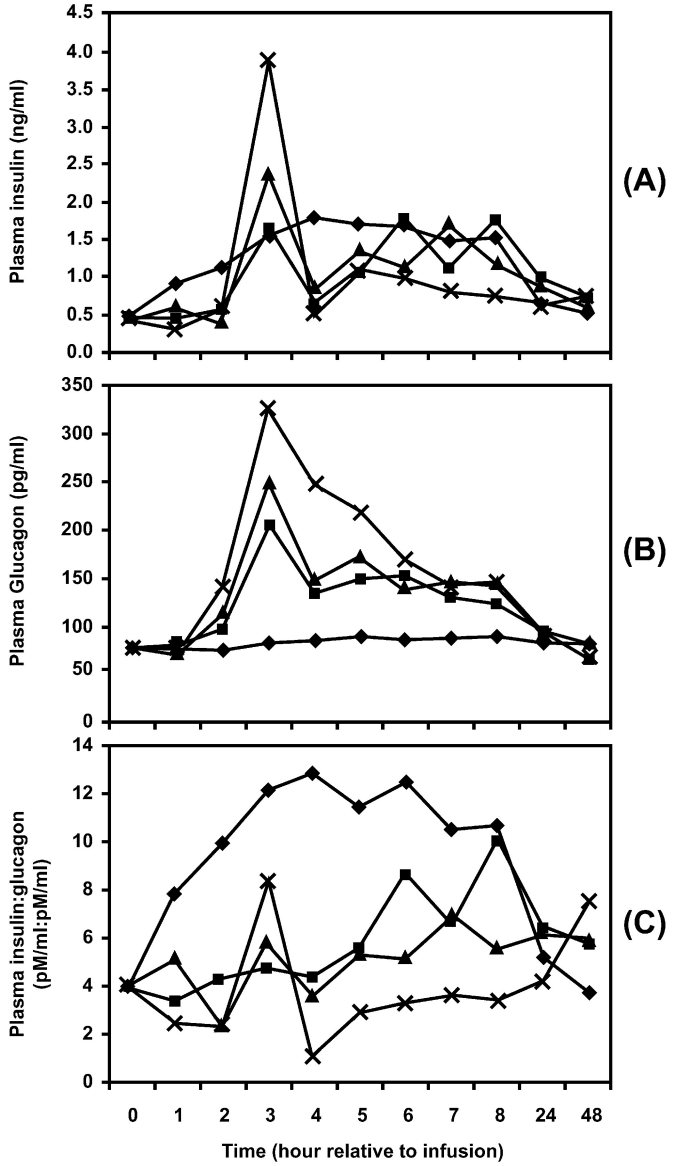

Figure 6. Plasma insulin (Panel A; SE = 0.35)* and glucagon (Panel B; SE = 18.9)** concentrations and the molar ratio of insulin to glucagon (Panel C; SE = 1.69)† following 0.0 (diamond), 0.5 (square), 1.0 (triangle), or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. * Treatment × time effect, $P = 0.007$; ** treatment × time effect, $P = 0.001$; linear effect, $P = 0.01$; †treatment × time effect, $P = 0.01$; linear effect, $P = 0.001$.

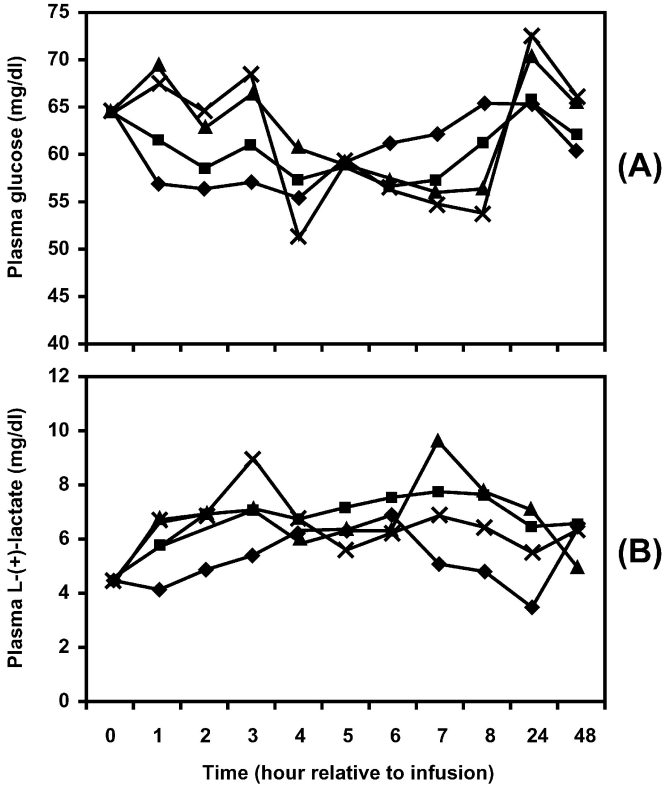

Figure 7. Plasma glucose (Panel A; SE = 3.3)* and L-(+)-lactate (Panel B; SE = 0.89)** concentrations following 0.0 (diamond), 0.5 (square), 1.0 (triangle), or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. *Treatment × time tendency, $P = 0.13$; **no effect of lipopolysaccharide, $P > 0.20$.

infusion were characterized by a peak in NEFA at 2 PIH, with values returning to control level by 3 PIH (Figure 11). Despite a tendency for elevated NEFA, plasma BHBA concentration decreased in a dose-dependent manner (treatment × time, $P = 0.003$; linear effect, $P = 0.04$) following intravenous LPS infusion (Figure 11). In vitro incubation of liver slices revealed a treatment-specific increase in the capacity (quadratic effect, $P = 0.02$) for the oxidation of [1-^{14}C]palmitate to CO_2. Hepatic capacities for the conversion of palmitate to acid-soluble ($P > 0.20$) or esterified products ($P = 0.18$) were not altered by LPS infusion (Figure 12); however, caution should be used when interpreting these data due to the low level of replication in the metabolic capacity study.

DISCUSSION

Proinflammatory cytokines, such as TNF-α, are responsible for the anorectic behavior observed during sickness (Johnson, 1998), and likely were the cause of the decreased DMI reported in this study (Figure 1). The effect of intravenous LPS infusion on DMI was dramatic and may be responsible for some of the metabolic effects reported; however, LPS infusion had maximal effects on many metabolic measurements at 2 to 3 PIH. Because ruminal fermentation continues for more than 48 h following feed withdrawal (Bergman, 1990), it is unlikely that decreased DMI dramatically affected metabolism in this short term (2 to 3 PIH). Additionally,

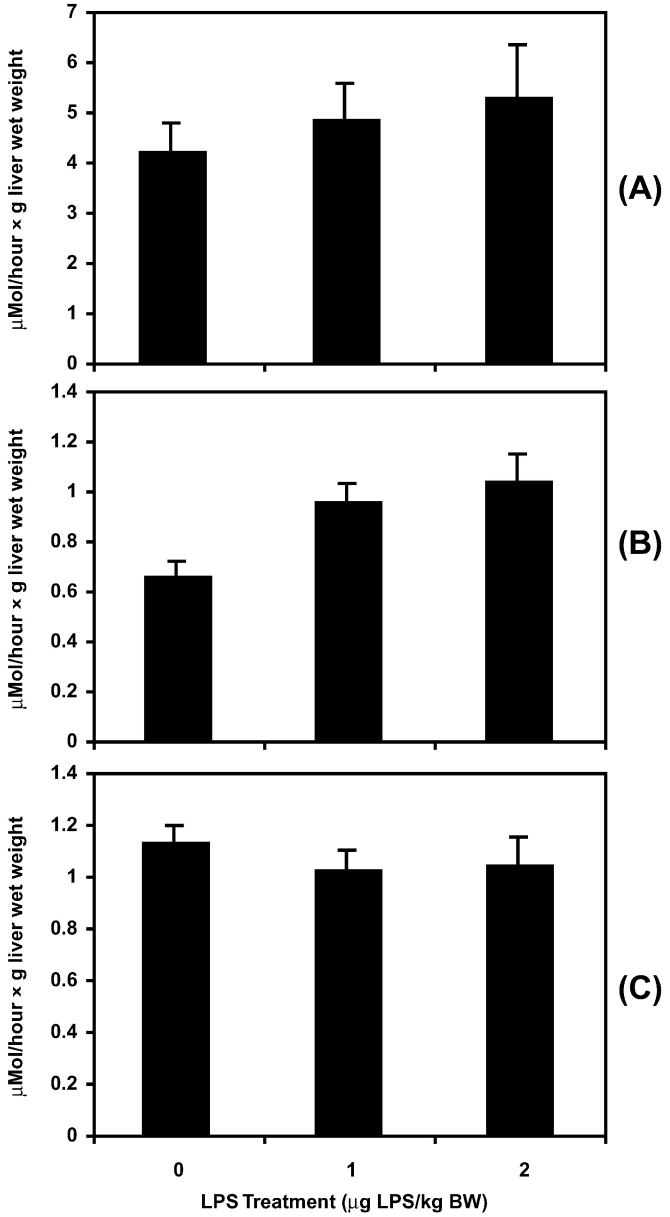

Figure 8. In vitro oxidation of [1-^{14}C]propionate (Panel A)*, [1-^{14}C]L(+)-lactate (Panel B)**, and [1-^{14}C]L-alanine (Panel C)* to CO_2 by liver slices following 0 (n = 6), 1 (n = 4), or 2 (n = 2) μg of intravenous lipopolysaccharide (LPS) infusion per kilogram of BW into midlactation dairy cows. *No effect of lipopolysaccharide, $P > 0.20$; **linear effect, $P = 0.01$.

during the study of liver metabolism, control animals were pair fed with a LPS-treated animal before liver biopsy; therefore, effects on metabolism were independent of feed intake.

It is not clear from the present study whether the negative effects of LPS infusion on milk production (Figure 2) were due to decreased DMI or an alternative mechanism; however, the above argument against the

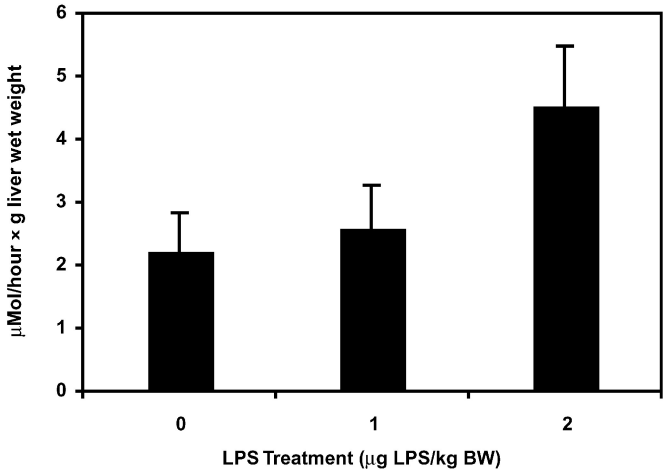

Figure 9. In vitro conversion of [1-^{14}C]propionate to glucose* by liver slices following 0 (n = 6), 1 (n = 4) or 2 (n = 2) μg of intravenous lipopolysaccharide (LPS) infusion per kilogram of BW into midlactation dairy cows. *Linear trend, $P = 0.07$.

effects of DMI on metabolism also persists for changes in milk production. Indeed, this reasoning is supported by Shuster et al. (1991b), who reported that LPS-induced hypogalactia occurred in the absence of changes in DMI. Furthermore, this hypogalactia reportedly is the result of systemic rather than local factors (Shuster et al., 1991a) and is not solely due to increased levels of circulating plasma cortisol (Shuster and Harmon, 1992) or changes in the GH/IGF-1 axis (Shuster et al., 1995). Instead, inflammatory cytokines such as TNF-α have been implicated as modulating milk production and secretion (Shuster et al., 1995).

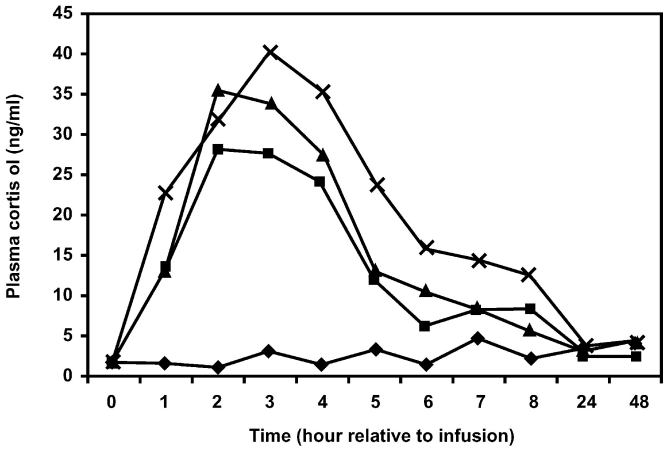

Figure 10. Plasma cortisol (SE = 3.3)* concentrations following 0.0 (diamond), 0.5 (square), 1.0 (triangle) or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. *Treatment × time effect, $P = 0.001$; linear effect, $P = 0.001$.

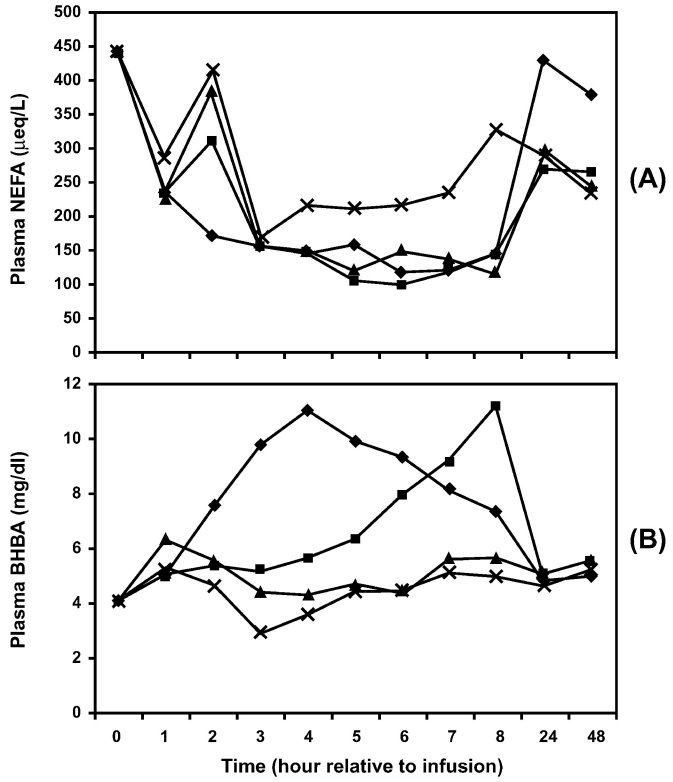

Figure 11. Plasma nonesterified fatty acids (NEFA; Panel A; SE = 61)* and β-hydroxybutyrate (BHBA; Panel B; SE = 1.1)** concentrations following 0.0 (diamond), 0.5 (square), 1.0 (triangle), or 1.5 (X) μg of intravenous lipopolysaccharide infusion per kilogram of BW into midlactation dairy cows. Means were adjusted by analysis of covariance. *Quadratic tendency, $P = 0.13$; **treatment × time effect, $P = 0.003$; linear effect, $P = 0.04$.

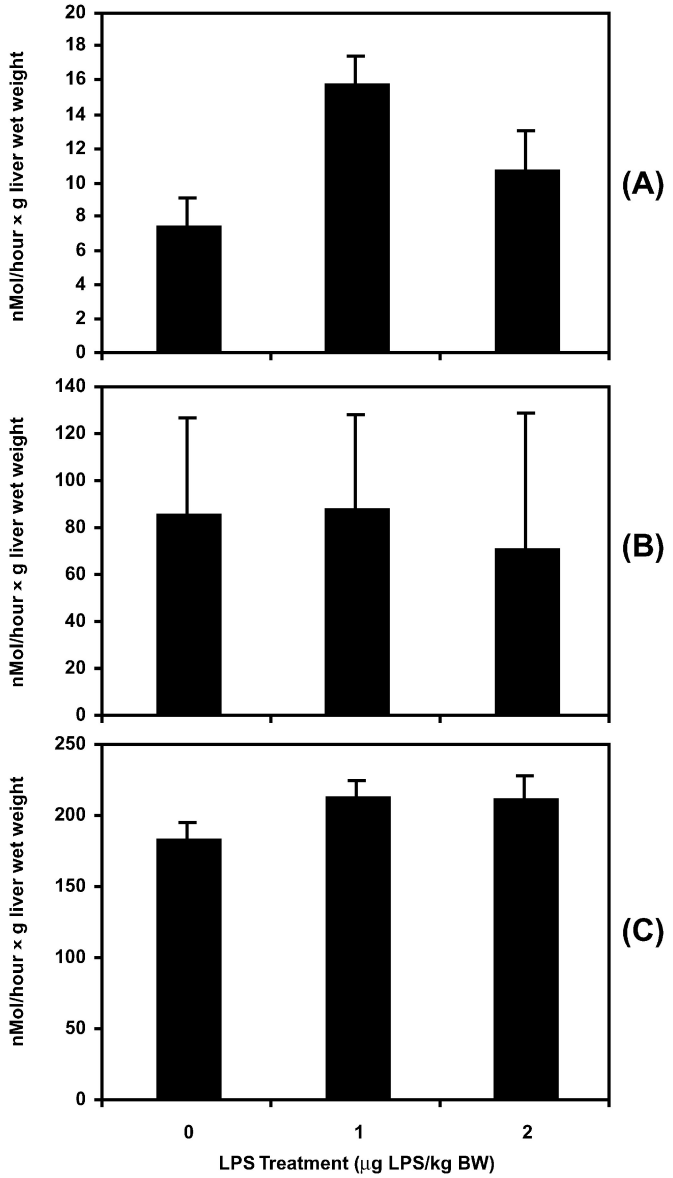

Figure 12. In vitro oxidation of [1-^{14}C]palmitate to CO_2 (Panel A)*, and conversion to acid-soluble products (Panel B)** or esterified products (Panel C)† by liver slices following 0 (n = 6), 1 (n = 4) or 2 (n = 2) μg of intravenous lipopolysaccharide (LPS) infusion per kilogram of BW into midlactation dairy cows. *Quadratic effect, $P = 0.02$; **no effect of lipopolysaccharide, $P > 0.20$; †no effect of lipopolysaccharide, $P = 0.18$.

The effects of LPS infusion on respiration rate (Figure 3, Panel A) were expected and typical of the labored breathing exhibited during sickness. Fever is also common during sickness or following LPS administration and is part the animal's controlled response to enhance immunological responses, limit pathogen growth, and aid in survival (Kluger et al., 1975). Although LPS induced fever in this study, cows receiving the greatest amount of LPS did not have the greatest increase in rectal temperature (Figure 3, Panel B). This quadratic effect and the large amount of variation reported in rectal temperature may have been due to the presence of barn fans; we observed that some individual cows administered with the 1.5 μg of LPS/kg of BW dose actually had rectal temperatures decrease to as low as 36.9°C when the fans were active.

In addition to the clinical signs discussed above, the increase in plasma TNF-α concentration (Figure 4) and dramatically altered circulating leukocyte populations reported in a companion paper for this study (Nonnecke et al., 2001) are evidence of immune activation achieved by LPS infusion. Because these measurements were unaffected by infusion of saline, immune activation can be attributed solely to the effects of LPS. Proinflammatory cytokines, such as TNF-α, are known to cause many changes in immune, endocrine, and metabolic tissues (Johnson et al., 1997). Because TNF-α was the first among assayed variables to be affected by LPS infusion, it may be responsible for many of the changes reported here and in companion papers (Nonnecke et

al., 2001; Waldron et al., 2003). Although this role is consistent with data in the literature implicating the proinflammatory cytokines (including TNF-α) in the perturbation of metabolism and productive processes following immune activation (Klasing, 1988; Johnson, 1997; Spurlock 1997), other endocrine and neuroendocrine modulators may also be involved.

Administration of LPS did not affect circulating GH and IGF-1 concentrations (Figure 5, Panels A and B, respectively). Shuster et al. (1995) reported that experimental *E. coli* mastitis in lactating dairy cattle resulted in GH resistance or an apparent uncoupling of the somatotropic axis (i.e., increased serum GH concentrations were not accompanied by changes in serum IGF-1 concentrations). Spurlock (1997) also reported uncoupling of the somatotropic axis in immune-challenged pigs such that administration of exogenous somatotropin did not prevent reductions in circulating IGF-1 concentrations. Investigations of immune-triggered uncoupling of the somatotropic axis in laboratory animals suggest that cytokines alter GH receptor signaling and subsequent expression of the acid-labile subunit of IGF-1 (Mao et al., 1999; Boisclair et al., 2000). Another aspect of the somatotropic axis that was not investigated here, but that may influence trophic activities, is the concentration of IGF binding proteins after immune activation. Changes such as those reported in calves (Elsasser et al., 1995) and sheep (Briard et al., 2000) could affect the metabolic influence of IGF-1 in a tissue-specific manner, even without changes in plasma IGF-1 concentrations. Davis (1998) postulated that uncoupling of the somatotropic axis may indirectly play a role in the immune response by partitioning nutrients away from productive tissues (e.g., skeletal muscle or mammary gland) for subsequent use by the immune system, and directly by positive actions of GH on the immune system. Indeed, GH has been shown to enhance immune function in healthy and diseased cows (reviewed by Burvenich et al., 1999).

The lack of effect of immune activation on plasma leptin concentration (Figure 5, Panel C) is contrary to reports of increased plasma leptin in rodents following LPS administration (Grunfeld et al., 1996; Sarraf et al., 1997; Roelfsema et al., 2001). Infusion of LPS into pigs resulted in either no change or decreased leptin mRNA expression in adipose tissue (Spurlock et al., 1998; Leininger et al., 2000) following immune activation. Our results are consistent with a report in sheep in which plasma leptin concentrations were unaffected by LPS administration (Soliman et al., 2001). Furthermore, plasma leptin concentrations were not different in nematode-infected heifers developing through 50 wk of age vs. those treated with an antiparasitic agent (Diaz-Torga et al., 2001). These reports suggest that plasma leptin is not responsible for the anorexia associated with chronic infection in ruminant species.

Plasma concentrations of insulin, glucagon, and ratios of insulin:glucagon were altered following LPS administration (Figure 6). It is not clear what effected these dramatic endocrine changes. However, changes in glucoregulatory hormones without a prior change in energy metabolites has been reported after immune activation in other species (Cornell, 1983). In some species, cytokines (Eizirik et al., 1995; Andersson et al., 2001) and other inflammatory mediators, such as nitric oxide (Southern et al., 1990; Spinas, 1999), affect the capacity of the pancreas to produce and secrete these hormones. Therefore, the maximal TNF-α concentrations at 2 PIH may have produced changes in plasma insulin and glucagon. Glucoregulatory hormone concentrations also may have been attributable to early changes in plasma glucocorticoid concentrations or potential sympathetic neuroendocrine changes.

The decreased molar ratio of insulin:glucagon, the lack of change in plasma glucose concentrations (Figure 7, Panel A), and the striking increase in plasma cortisol concentrations (Figure 10) all indicate an increased gluconeogenic environment following LPS administration. These measurements are supported by data in Figure 9 showing a tendency for increased capacity of liver slices to convert [1-^{14}C]propionate to glucose following LPS infusion. These data are similar to those reported by Naylor and Kronfeld (1985) in sheep. In contrast, immune activation in rodents increased plasma glucagon and insulin concentrations resulting in decreased hepatic gluconeogenesis (Filkins and Buchanan, 1977). In the study by Naylor and Kronfeld (1985), an initial dramatic increase in net glucose release, followed by a moderate but still elevated hepatic glucose release were observed. The initial increase in hepatic glucose release was likely the result of enhanced glycogenolysis, whereas the sustained increased production of glucose was from gluconeogenesis after the depletion of hepatic glycogen. The present study is the first to assess gluconeogenic capacity in dairy cattle challenged with LPS. In cows (Lohuis et al., 1988) and heifers (Werling et al., 1996; Steiger et al., 1999), plasma concentrations of glucose following endotoxin or LPS administration increased initially and then decreased after several hours. These groups proposed an initial increase in glycogenolysis and gluconeogenesis followed by a subsequent increase in peripheral glucose utilization and decrease in gluconeogenesis similar to that reported for other species. These apparent inconsistencies in the literature and the importance of gluconeogenesis to the lactating dairy cow necessitate further investigation of glucose kinetics, especially during early lactation.

Immune activation by LPS results in hyperlactemia in most nonruminant species (Mizock, 1995) and in sheep (Naylor and Kronfeld, 1985). In the latter study, hyperlactemia was accompanied by increased hepatic uptake of lactate that could have maximally accounted for 50% of the hepatic glucose production during the phase when hepatic glycogen stores were likely depleted. Giri et al. (1990) reported that hyperlactemia occurred when 2.5 μg/kg, but not 1.0 μg/kg of LPS was administered intravenously to pregnant, nonlactating cows. This report is consistent with our data indicating no effect of lower-dose LPS administration on plasma lactate concentration (Figure 7, Panel B). Interestingly, LPS administration did increase the capacity of liver slices to oxidize [1-^{14}C]L-lactate to CO_2 in this study (Figure 8, Panel B). Although this increased oxidation could contribute to reducing equivalents in support of immune function, it is of further interest to determine the contribution of lactate to gluconeogenesis during inflammatory states.

Although the in vitro conversion of [1-^{14}C]L-lactate to CO_2 was increased during immune activation, the conversion of [1-^{14}C]alanine to CO_2 was unchanged (Figure 8, Panel C). Both lactate and alanine enter the oxidative pathway through a common intermediate (pyruvate). Reasons why oxidation of alanine was unaffected by immune activation may come from Meinz et al. (1998), who investigated hepatic uptake of gluconeogenic AA in endotoxic dogs. They reported that although the fractional extraction of glycine, serine, threonine, and glutamine increased following endotoxin administration, fractional extraction of alanine was unchanged. They postulated that a specific defect in hepatic AA transporters might occur whereby gluconeogenic AA other than alanine are transported via an endotoxin- or cytokine-stimulated mechanism. Although the data of Meinz et al. (1998) remain to be substantiated in the cow, impaired alanine transport into hepatocytes following LPS administration might explain the unchanged alanine oxidation, despite the apparent upregulation of oxidative metabolism.

The current study is the first to document the in vitro hepatic conversion of radiolabeled palmitate to CO_2, acid-soluble products, and esterified products (Figure 12) in ruminants following immune activation. The increased hepatic oxidation of palmitate may provide reducing equivalents for acute-phase protein synthesis by hepatocytes; additionally, the NADPH produced might be used for the generation of reactive oxygen species and the maintenance of the glutathione cycle during the inflammatory response (Spitzer, 1995). Furthermore, the apparent shunting of fatty acids toward oxidation instead of esterification, during a period of increased NEFA presentation to the liver, may prevent the accumulation of triglycerides in the liver.

Reports of plasma NEFA levels in ruminants following endotoxin administration are contradictory (sheep, Naylor and Kronfeld, 1986; cattle, Steiger et al., 1999); however, increased levels of glucocorticoids and catecholamines, and depressed feed intake would be consistent with increased circulating NEFA levels. Furthermore, Kushibiki et al. (2002) reported that intravenous recombinant TNF-α increased plasma NEFA concentrations without affecting DMI in heifers. These concepts are consistent with our data showing a tendency for increased plasma NEFA concentrations following LPS infusion into midlactation cows (Figure 11, Panel A).

In another aspect of lipid metabolism, we observed a marked decrease in circulating BHBA levels following LPS administration in this study (Figure 11, Panel B). The liver (during periods of negative energy balance) and the ruminal epithelium (in well-fed ruminants) are the main sources of BHBA in dairy cattle. Most of the circulating BHBA in well-fed midlactation cows is likely a result of butyrate metabolism in the ruminal epithelium. Assuming ruminal epithelium was the primary ketogenic tissue in cows during this study, it is not clear whether the decreased release of BHBA by the ruminal epithelium was the result of decreased ketogenic capacity of the tissue or whether substrate supply (i.e., butyrate) was limiting due to decreased DMI following LPS infusion. The former concept is supported by data indicating that endotoxin administration reportedly decreased hepatic ketogenesis in mice (Memon et al., 1992) and decreased circulating BHBA concentrations in LPS-infused cattle relative to their pair-fed controls (Werling et al., 1996; Steiger et al., 1999). The idea of an antiketogenic mechanism following immune activation is intriguing, but requires further study. Preliminary data generated using in vitro liver-slice incubations in our laboratory suggest that hepatic ketogenic capacity of cattle, as indicated by acid soluble product accumulation, is not altered following LPS infusion (Figure 12, Panel B). However, as previously noted, none of the cows on this study likely had significant rates of hepatic ketogenesis before LPS administration; therefore, the potential of immune activation to inhibit in vitro hepatic ketogenic capacity may have been minimal. From a teleological standpoint, ketogenesis should yield an alternate energy source that could be used by tissues at a time when glucose use by the immune system is of high priority. Moderate ketogenesis would therefore seem to benefit the immune-challenged host. However, an antiketogenic effect in the immune-challenged cow could minimize the negative effects of keto-

sis on immune function (as reviewed by Suriyasathaporn et al., 2000).

The activation of leukocytes, production of communication and signaling molecules by immune and nonimmune tissues, and production of supporting compounds, such as acute-phase protein synthesis by the liver, represent a significant energy and metabolite cost to the animal following immune activation. These processes were supported by relatively minor changes in the circulating concentrations of most plasma energy metabolites and dramatic changes in DMI and milk production following LPS administration. Coordination of productive, metabolic, and immune functions was achieved through autocrine, paracrine, and endocrine control via factors including cytokines, insulin, glucagon, cortisol, GH, IGF-1, and, perhaps, other endocrine mediators. Increased hepatic capacity to for oxidation of L-(+)- lactate and palmitate to CO_2, and the trend for increased gluconeogenic capacity from propionate by liver slices are examples of changes in tissue metabolism to support homeostasis following LPS administration. As suggested by Spurlock (1997), this integrated coordination of tissue and systemic regulation in support of the immune response should be considered a homeorhetic state allowing downregulated, yet successful, maintenance of a physiologic state such as lactation and/or pregnancy. Furthermore, these homeorhetic changes may have important implications for metabolic health during an immune challenge and warrant further investigation.

CONCLUSIONS

Immune activation by intravenously administered LPS resulted in significant dose-dependent changes in productive, clinical, and metabolic measurements in midlactation dairy cows. In vivo and in vitro data indicate that aspects of energy metabolism important to metabolic health are altered following immune activation and suggest possible mechanisms for the associative relationship between infectious disease and metabolic disorders in dairy cattle.

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