

# Estradiol/progesterone implants increase food intake, reduce hyperglycemia and increase insulin resistance in endotoxic steers

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## Abstract

High doses of lipopolysaccharide (LPS) induce transient hyperglycemia, then chronic hypoglycemia and increased insulin resistance. In addition, appetite is reduced, while body temperature and concentrations of cortisol and tumor necrosis factor alpha (TNF $\alpha$ ) are elevated. Furthermore, concentrations of GH and IGF-I are reduced in cattle. The objectives of this study were to determine whether a gonadal steroid implant (20 mg estrogen and 200 mg progesterone) given to endotoxemic steers would: (1) reduce hyperglycemia, reduce hypoglycemia, reduce insulin resistance, (2) reduce changes in concentrations of GH and IGF-I, (3) reduce inappetence and reduce concentrations of blood urea nitrogen (BUN) and non-esterified fatty acids (NEFA), and (4) reduce fever and concentrations of TNF $\alpha$  and cortisol. Holstein steers were assigned within a 2  $\times$  2 factorial arrangement of treatments as follows ( $n=5$  per group): C/C, no steroid and vehicle; S/C, steroid and vehicle; C/E, no steroid and LPS (1  $\mu$ g/kg body weight (BW), i.v.); S/E, steroid and endotoxin. Steroid implants were given at 20 weeks of age (day 0) and serial blood samples (15 min) were collected on day 14 for 8 h, with vehicle or LPS injected after 2 h. Intravenous glucose tolerance tests (100 mg/kg BW) were carried out at 6 h and 24 h. Hyperglycemia was 67% lower ( $P<0.05$ ) in S/E- compared with C/E-treated steers between 30 and 150 min after i.v. injection of LPS.

Hypoglycemia developed after 4 h and insulin resistance was greater in S/E- compared with C/E-treated steers ( $P<0.05$ ) at 6 and 24 h. Concentrations of IGF-I were restored earlier in steroid-treated steers than in controls. Concentrations of GH were not affected by steroids, but increased 1 h after injection of LPS, then were reduced for 2 h. Appetite was greater ( $P<0.05$ ) in S/E- (2.1% BW) compared with C/E-treated steers (1.1% BW) (pooled s.e.m.=0.3). Concentrations of NEFA increased after injecting LPS, but concentrations were lower ( $P<0.05$ ) in S/E- compared with C/E-treated steers. LPS did not affect concentrations of BUN, but concentrations were lower in steroid-treated steers. Steroids did not affect body temperature or concentrations of TNF $\alpha$  and cortisol. In summary, gonadal steroids reduce hyperglycemia, reduce inappetence and tissue wasting, but increase insulin resistance. Furthermore, concentrations of IGF-I are restored earlier in steroid-treated than in non-steroid-treated steers injected with LPS. It is concluded that gonadal steroids reduce severity of some endocrine and metabolic parameters associated with endotoxemia. However, it is unlikely that gonadal steroids acted via anti-inflammatory and immunosuppressive actions of glucocorticoids or through reducing concentrations of cytokines.

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## Introduction

Administration of lipopolysaccharide (LPS), a structural component in the outer cell wall of Gram-negative bacteria, stimulates an immune response in mammalian hosts which leads to reduced appetite, altered metabolism and a reduced growth rate or loss of body weight (Coleman *et al.* 1993, Elsasser *et al.* 1995, Heath *et al.* 1997). Concentrations of growth hormone (GH) and insulin-like growth factor-I (IGF-I) are reduced in cattle after an injection of LPS (Elsasser *et al.* 1995). Further-

more, glucose homeostasis is severely disrupted; low doses of LPS induce hyperglycemia between 1 to 3 h, while higher doses of LPS induce chronic hypoglycemia, which develops after initial hyperglycemia and may persist for days (Lang *et al.* 1985, Giri *et al.* 1990, Elsasser *et al.* 1996). In addition, animals become insulin resistant, which, coupled with hypoglycemia, can be fatal because insufficient glucose is available for basal tissue requirements (Cryer 1992).

A number of studies have suggested that anabolic agents assist recovery from catabolic states (Clemmons *et al.* 1992,

Byrne *et al.* 1993, Elsasser *et al.* 1994, Zhao & Donovan 1995, Heath *et al.* 1997). In addition, gonadal steroid implants improve growth rates, enhance accretion of lean tissue and improve the conversion efficiency of feed in healthy steers (Unruh 1986, Breier *et al.* 1988, Enright *et al.* 1990) and this may be beneficial during catabolic disease. During infection, gonadal steroids are immunomodulatory, but their role in host defense is not well understood; actions range from increased severity of infection to enhanced recovery from infection (Grossman 1984, 1985, 1994). However, subcutaneous implants containing estradiol (E<sub>2</sub>) and progesterone (P<sub>4</sub>) reduce severity and enhance recovery from coccidiosis in steers (Heath *et al.* 1997). In that study, implanted steers ate more, did not lose weight and had a shorter duration of fever compared with infected, but non-implanted steers and there was an increased percentage of lymphocytes expressing CD4 antigens. Therefore, gonadal steroids may provide protection against infection and disease in addition to their actions on growth.

The hypothesis of the present study is that steroid implants would reduce the severity of endotoxemia in steers injected i.v. with a high dose of LPS. The objectives of this study were to determine whether E<sub>2</sub>/P<sub>4</sub> implants would: first, reduce hyperglycemia, reduce hypoglycemia and reduce insulin resistance; secondly, reduce changes to concentrations of GH and IGF-I; thirdly, increase food intake and decrease mobilization of body tissues; and fourthly, reduce duration of fever and concentrations of tumor necrosis factor alpha (TNF $\alpha$ ) and cortisol.

## Materials and Methods

### Animals and maintenance

Twenty Holstein steers obtained from the EV Smith Research Center (Shorter, AL, USA) were hand-reared indoors on whole milk. After weaning between 7 and 9 weeks of age, steers were released onto pasture and fed a concentrate feed which was calculated to provide weight gain of 1 kg/day (14.9% dietary crude protein). Steers were free to graze and had access to water at all times.

### Experimental design

Steers were allocated at random into one of four treatment groups ( $n=5$  per group) in a  $2 \times 2$  factorial arrangement as follows: C/C, no steroid and vehicle (saline control); S/C, steroid (20 mg E<sub>2</sub> and 200 mg P<sub>4</sub>, synovex-s, Fort Dodge, Des Moines, IO, USA) and vehicle; C/E, no steroid and given LPS i.v. (1  $\mu$ g/kg body weight (BW) in saline (0.1 mg/ml) (*E. coli* serotype 055:B5, Sigma Chemical Corporation, St Louis, MO, USA)); S/E, steroid and LPS. At 20 weeks of age (day 0), E<sub>2</sub>/P<sub>4</sub> was implanted in an ear of each S/C and S/E steer in accordance with manufac-

turer's instructions. Mean BW was  $168.1 \pm 3.7$  kg and average daily gain (ADG) was  $790 \pm 277$  g/day. Neither BW nor ADG significantly differed ( $P>0.05$ ) between groups. One week later (day 7), steers were moved from pasture to individual pens indoors. While indoors, steers were fed daily at 10% surplus to demand and had free access to feed. Feed intake was recorded daily and converted to dry matter intake (DMI) after correcting for moisture content by oven drying feed and refusal sub-samples at 50 °C for 48 h. Water was freely available.

Rectal temperature was monitored and jugular blood samples were collected from day 11. A jugular vein of each steer was cannulated on day 13 and on the morning of day 14, feed was removed at 0800 h and at 1000 h blood samples (3 ml) were collected at 15 min intervals for 2 h, then LPS was injected i.v. and blood samples were collected at 15 min intervals for 6 h. Intravenous glucose tolerance tests (IVGTTs) were carried out at 6 and at 24 h after injection of LPS to assess glucose tolerance during hypoglycemia and during recovery from endotoxemia. Glucose (50% Dextrose Injection, Baxter Healthcare, Deerfield, IL, USA) was injected i.v. into all steers (100 mg/kg BW) immediately after the 8 h sample was collected and blood samples were collected for a further hour at 5, 10, 15, 30, 45 and 60 min. This protocol was repeated 24 h later with three blood samples collected at 15 min intervals before i.v. injection of glucose. Blood was collected into tubes containing EDTA (7.5 mg) and kept on ice before centrifugation within 15 min of collection. One aliquot of harvested plasma was stored at  $-20$  °C and a second aliquot was stored at  $-70$  °C for assaying non-esterified fatty acids (NEFA). For behavioral indications of stress, the duration of three pre-determined factors (panting; coughing or grunting; and saliva drool) were recorded at 15 min intervals for 7 h following injection of LPS.

Studies were done from February to May and all experimental procedures were approved by the Institutional Animal Care and Use Committee at Auburn University.

### Hormone and metabolite assays

Insulin was assayed using a commercial RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Parallelism was demonstrated, curve displacement averaged 92.8% over five different concentrations, spiked recovery averaged 99.4% over four different concentrations, and the sensitivity was 15.8 pmol/l. Intra- and interassay coefficients of variation (CVs) were 3.7 and 3.3% respectively. Cortisol was assayed in a modified kit from Diagnostic Products Corporation using hydrocortisone (Sigma) as standard, which was prepared in charcoal-stripped steer plasma as previously described (Sartin *et al.* 1988b). Standard curve concentrations ranged

from 0.78 to 100 ng/ml. Intra- and interassay CVs were 4.3 and 17.2% respectively.

GH was assayed as previously described (Sartin *et al.* 1985). Intra- and interassay CVs were 9.5 and 14.1% respectively. IGF-I was assayed in one assay as previously described (Elsasser *et al.* 1988). The intra-assay CV was 9.8%. TNF $\alpha$  was assayed in one assay as previously described (Kennison *et al.* 1990). The intra-assay CV was 8.7%. Glucose, blood urea nitrogen (BUN) and NEFA were assayed in modified colorimetric kits (glucose and BUN from Sigma and NEFA from Wako Chemicals, Richmond, VA, USA). Briefly, samples were diluted (1:20 (glucose and BUN) or 1:2 (NEFA)), assayed in duplicate and read in a spectrophotometer, at wavelengths specified by the manufacturers, against a linear standard curve (range 6.26–400 mg/dl (glucose); 2.34–150 mg/dl (BUN); and 0.0156–1.97 mEq/l (NEFA)) in 96-well ELISA plates. Intra- and interassay CVs were 5.7 and 3.4% respectively for glucose, 1.8 and 3.3% respectively for BUN and 8.6 and 13.9% respectively for NEFA.

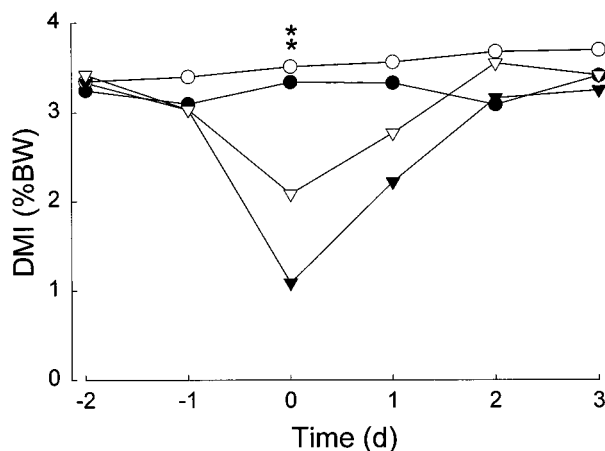
#### Statistical analysis

Pulse secretion parameters of GH (mean, smoothed mean, peak amplitude, peak interval and inter-peak interval) were determined from 0 to 8 h using the Pulsar pulse detection algorithm (Merriam & Wachter 1982). We have previously described detection criteria for Pulsar (Sartin *et al.* 1988b). Glucose half-life ( $t_{1/2}$ ) was determined from log-transformed glucose decay curves from 0 to 45 min (Gibaldi & Perrier 1982). Total area under the insulin curve was calculated from 0 to 45 min using the trapezoid method, then baseline was calculated as the area of the rectangle projected from 0 to 45 min and deducted from the total area to give a net area under the curve (insulin AUC). Similarly, net AUCs for glucose and GH were calculated from 0–150 min and 120–180 min respectively.

Data were subjected to ANOVA using the generalized linear models procedure in SAS (1990). Data for pulse secretion parameters of GH, insulin AUC, glucose AUC, GH AUC and  $t_{1/2}$  for glucose were analyzed as a 2  $\times$  2 factorial arrangement of treatments with E<sub>2</sub>/P<sub>4</sub> implant or no implant and LPS or saline as factors. In addition, data for DMI, NEFA, BUN, GH, glucose, insulin, TNF $\alpha$ , cortisol and body temperature were subjected to repeat measures ANOVA with the factors detailed above. When treatments were significant, least-squares means were compared using *t*-tests.

## Results

Feed intake was reduced on day 0 ( $P < 0.001$ ) and day 1 ( $P < 0.01$ ) in LPS-treated compared with non-LPS-treated groups (Fig. 1). In addition, S/E-treated steers ate more ( $P < 0.01$ ) than C/E-treated steers on day 0.



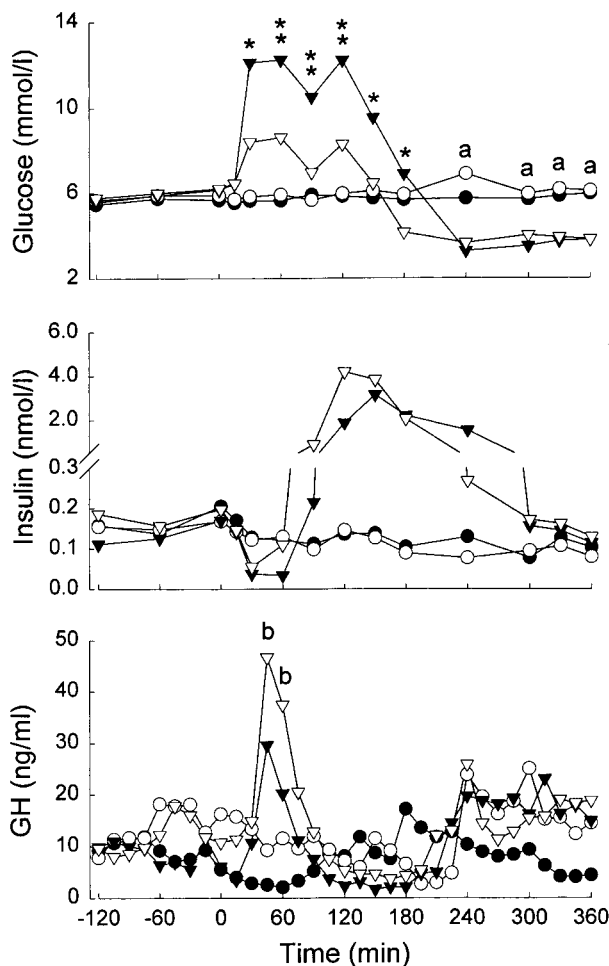
**Figure 1** Least squares mean DMI expressed as a percentage of BW 2 days before and 3 days after i.v. injection of vehicle or LPS (1  $\mu$ g/kg BW) on day 0. Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Asterisks denote a difference between S/E and C/E group means at day 0 (\*\* $P < 0.01$ ). Pooled s.e.m. = 0.3.

Concentrations of glucose were higher ( $P < 0.001$ ) in LPS-treated compared with non-LPS-treated groups from 0 to 150 min after i.v. injection of vehicle or LPS (Fig. 2). However, the glucose AUC was lower ( $P < 0.01$ ) in S/E-treated steers (218  $\text{mmol}\cdot\text{min}\cdot\text{l}^{-1}$ ) than C/E-treated steers (668  $\text{mmol}\cdot\text{min}\cdot\text{l}^{-1}$ ) during this period (pooled s.e.m. = 90). Mean concentrations of glucose declined in plasma of LPS-treated compared with non-LPS-treated after 180 min and remained lower ( $P < 0.001$ ) from 240 to 360 min.

Concentrations of insulin were not significantly different between groups before i.v. injection of vehicle or LPS (Fig. 2), but at 30 min concentrations of insulin were lower ( $P < 0.001$ ) in LPS-treated (46.5 pmol/l) compared with non-LPS-treated (124.5 pmol/l) steers (pooled s.e.m. = 12.6) and remained lower at 60 min ( $P < 0.05$ ). After 60 min, concentrations of insulin increased ( $P < 0.001$ ) in plasma of LPS-treated compared with non-LPS-treated steers and the insulin AUC from 60 to 300 min was not significantly different between C/E- and S/E-treated steers (Fig. 2).

Glucose  $t_{1/2}$  was shorter ( $P < 0.05$ ) in LPS-treated compared with non-LPS-treated steers at the 6 h IVGTT, but was not significantly different between groups at the 24 h IVGTT (Table 1). Steroid treatment did not significantly affect glucose  $t_{1/2}$  at either 6 or 24 h and there were no significant interactions between LPS and steroid treatments.

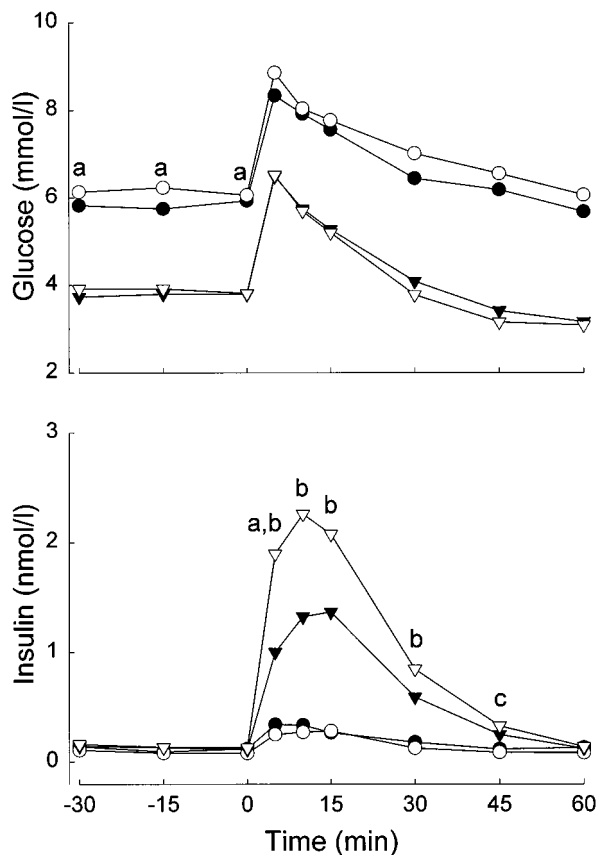
Concentrations of insulin were not significantly different between groups before IVGTT at 6 h. However, concentrations of insulin were higher ( $P < 0.05$ ) in S/E-treated steers than other treatment groups before IVGTT



**Figure 2** Least squares mean concentrations of glucose, insulin and GH in plasma 120 min before and for 360 min after i.v. injection of vehicle or LPS (1 µg/kg BW) at 0 min. Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Letters denote differences between S/E and C/E group means (<sup>a</sup> $P < 0.05$ ; <sup>\*\*</sup> $P < 0.01$ ). Letters denote differences between LPS- and non-LPS-treated group means (<sup>a</sup> $P < 0.001$ ; <sup>b</sup> $P < 0.1$ ). Pooled s.e.m. were 0.6 for glucose, 0.3 for insulin and 4.7 for GH.

at 24 h. Insulin AUC after each IVGTT (6 and 24 h) was greater in LPS-treated than in non-LPS-treated groups (Figs 3 and 4; Table 1). In addition, insulin AUC had declined at 24 h compared with 6 h, but remained higher in LPS-treated compared with non-LPS-treated steers. Furthermore, insulin AUC was greater in S/E- than in C/E-treated steers at both 6 h and 24 h IVGTT (Table 1).

A synchronous increase ( $P < 0.1$ ) in concentrations of GH occurred between 30 and 90 min in LPS-treated compared with non-LPS-treated steers (Fig. 2). GH AUC from 120 to 180 min was lower ( $P < 0.01$ ) in LPS-treated ( $173 \text{ ng} \cdot \text{min} \cdot \text{ml}^{-1}$ ) than non-LPS-treated groups



**Figure 3** Least squares mean concentrations of glucose and insulin 30 min before and for 60 min after i.v. injection of glucose (100 mg/kg BW) given 6 h after i.v. injection of vehicle or LPS (1 µg/kg BW). Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Letters denote differences between LPS- and non-LPS-treated group means (glucose=<sup>a</sup> $P < 0.001$ ; insulin=<sup>b</sup> $P < 0.001$ ; <sup>c</sup> $P < 0.01$ ). In addition, insulin concentrations for S/E- and C/E-treated steers were significantly different 5 min after IVGTT (<sup>a</sup> $P < 0.05$ ). Pooled s.e.m. were 0.26 for glucose and 0.17 for insulin.

( $755 \text{ ng} \cdot \text{min} \cdot \text{ml}^{-1}$ ) (pooled s.e.m.=111). Regression analysis shows these reduced concentrations of GH to be highly related ( $P < 0.001$ ) to increased concentrations of insulin in LPS-treated compared with non-LPS-treated groups (equations not shown). Despite these fluctuations in concentrations of GH, pulse secretion parameters showing mean, smoothed mean and amplitude were higher in steroid-treated than in non-steroid-treated groups (Table 2).

Concentrations of IGF-I declined in plasma of LPS-treated compared with non-LPS-treated steers from day 0 until day 2 (Fig. 5). On day 3, concentrations of IGF-I were not significantly different between all groups. However, when S/E- and C/E-treated groups were considered

**Table 1** Least squares mean  $t_{1/2}$  of glucose in plasma and mean insulin AUC at 6 h and 24 h after i.v. injection of LPS (1  $\mu$ g/kg BW)

Group	Glucose $t_{1/2}$ (min)		Insulin net AUC (pmol·min·ml <sup>-1</sup> )	
	6 h	24 h	6 h	24 h
C/C	101.6 <sup>a</sup>	94.9	5933	9065
S/C	105.1 <sup>b</sup>	75.9	4139	7812
C/E	48.6 <sup>a,**</sup>	108.6 <sup>**</sup>	31 422 <sup>a,†</sup>	16 435 <sup>c,†</sup>
S/E	38.3 <sup>b,†</sup>	75.9 <sup>†</sup>	52 115 <sup>a,**</sup>	27 319 <sup>c,**</sup>

Pooled S.E.M. for glucose and insulin was 14.2 and 5543 respectively.

Like letters within columns denote differences between treatment means:

<sup>a,b</sup> $P < 0.01$ .

Like symbols within rows denote differences between treatment means:

<sup>†</sup> $P < 0.10$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ .

alone, concentrations of IGF-I were higher in S/E- ( $P < 0.05$ ) than in C/E-treated steers on day 3, but not different from S/C- or C/C-treated steers ( $P > 0.05$ ).

Concentrations of NEFA increased in LPS-treated compared with non-LPS-treated groups at 12 h ( $P < 0.001$ ) and remained higher than non-LPS-treated at day 1 ( $P < 0.05$ ) (Fig. 6). In addition, concentrations of NEFA were significantly lower in S/E- than in C/E-treated steers at 12 h.

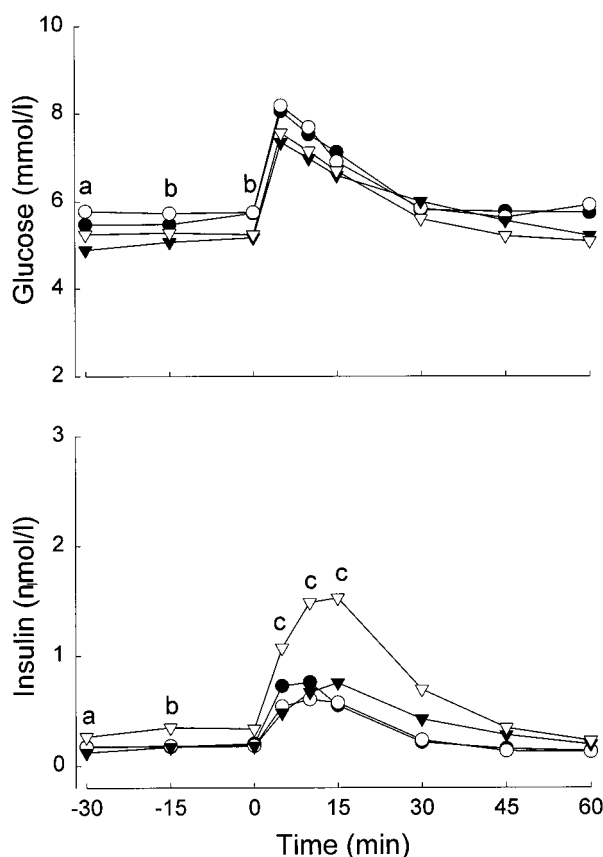
Concentrations of BUN were lower in plasma of steroid-treated compared with non-steroid-treated steers at all sample times (Fig. 6). In addition, concentrations of BUN were elevated in plasma of LPS-treated compared with non-LPS-treated steers at 12 h and on day 1.

Concentrations of TNF $\alpha$  increased ( $P < 0.001$ ) in LPS-treated compared with non-LPS-treated steers 30 min after i.v. injection of vehicle or LPS and were maximal in plasma at 60 min, then declined over the next 3 h (Fig. 7).

Body temperature increased ( $P < 0.05$ ) in LPS-treated compared with non-LPS-treated steers after 60 min with a maximum observed at 240 min, then declined over the remainder of the study (Fig. 7). Body temperature was not significantly different between LPS-treated groups.

Concentrations of cortisol increased ( $P < 0.001$ ) in LPS-treated compared with non-LPS-treated steers after 30 min and remained elevated until 240 min, then declined over the remainder of the study (Fig. 7). Concentrations of cortisol were not significantly different between LPS-treated groups.

Saliva drooled from the mouths of C/E-treated steers for  $41.3 \pm 5.7$  min and was not observed in other groups ( $P < 0.01$ ). In addition, duration of coughing and/or grunting was shorter ( $P < 0.01$ ) in S/E- (12 min) than in C/E-treated steers (109 min) (pooled S.E.M.=13). However, duration of panting was not significantly different between S/E- (278 min) and C/E-treated steers (252 min) (pooled S.E.M.=52).



**Figure 4** Least squares mean concentrations of glucose and insulin 30 min before and for 60 min following i.v. injection of glucose (100 mg/kg BW) given 24 h after i.v. injection of vehicle or LPS (1  $\mu$ g/kg BW). Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Letters denote differences between LPS- and non-LPS-treated group means (glucose=<sup>a</sup> $P < 0.01$ ; <sup>b</sup> $P < 0.05$ ). In addition, concentrations of insulin were significantly different between S/E and C/E groups (<sup>c</sup> $P < 0.05$ ). Pooled S.E.M. were 0.24 for glucose and 0.12 for insulin.

## Discussion

The results of the present study show that hyperglycemia is reduced, food intake is increased and less fat is mobilized in steers implanted with E<sub>2</sub>/P<sub>4</sub> during endotoxemia. In addition, behavioral distress was reduced and concentrations of IGF-I were restored earlier in steers implanted with E<sub>2</sub>/P<sub>4</sub> during endotoxemia. Furthermore, concentrations of BUN were lower in E<sub>2</sub>/P<sub>4</sub>-implanted steers throughout the study, indicating that protein was spared. Collectively, these data support the hypothesis that E<sub>2</sub>/P<sub>4</sub> implants reduce severity of endotoxemia. However, hypoglycemia was not affected and insulin resistance increased in steers implanted with E<sub>2</sub>/P<sub>4</sub> during endotoxemia. Therefore, E<sub>2</sub>/P<sub>4</sub> implants do not affect all aspects of glucose homeostasis.

**Table 2** Least squares mean GH pulse parameters assessed from blood samples collected over the 8 h period from 2 h before to 6 h after injection of vehicle or LPS

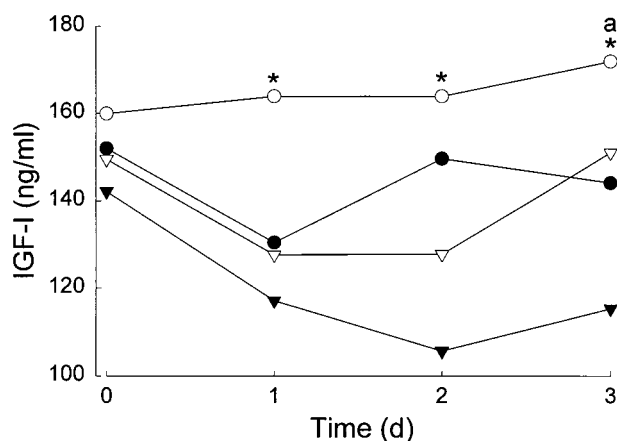
Group	Mean (ng/ml)	Smooth mean (ng/ml)	Amplitude (ng/ml)	Peak interval (min)	Inter-peak interval (min)
C/C	7.37 <sup>b</sup>	3.21 <sup>b</sup>	5.99 <sup>d</sup>	44.88	64.32
S/C	12.85 <sup>a</sup>	4.48 <sup>a</sup>	11.55 <sup>c</sup>	36.80	57.35
C/E	8.35 <sup>b</sup>	3.73 <sup>b</sup>	6.65 <sup>d</sup>	37.71	58.80
S/E	12.65 <sup>a</sup>	5.53 <sup>a</sup>	10.26 <sup>c</sup>	41.24	61.89
Pooled S.E.M.	1.95	0.68	2.14	4.85	5.42

Different letters within columns denote differences within columns between treatment means: <sup>a,b</sup> $P < 0.05$ ; <sup>c,d</sup> $P < 0.01$ .

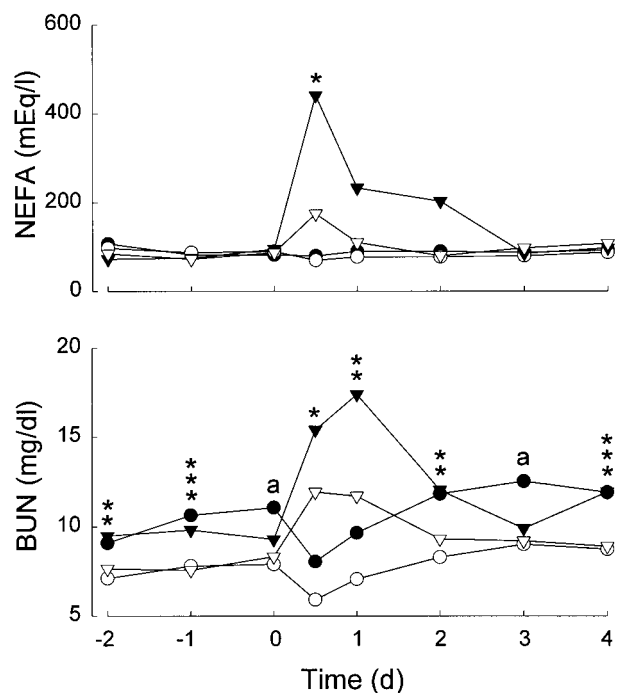
Hyperglycemia develops after injection of LPS because an increased rate of gluconeogenesis and increased release of glucose from the liver, together exceed increased tissue demand for glucose (Wolfe *et al.* 1977, Lang *et al.* 1985). Uptake of glucose increases in diaphragm, fat, ileum, kidney, liver, lung, skin and spleen – tissues rich in macrophages – but not muscle (Mészáros *et al.* 1988, Lang & Dobrescu 1991a,b, Lang *et al.* 1993). Decreased glucose requirements in skeletal muscle, but not cardiac muscle, during endotoxemia may spare glucose for increased antibacterial activity in organ tissues (Mészáros *et al.* 1988, Lang *et al.* 1993). It cannot be determined from the current study whether reduced hyperglycemia in S/E-treated steers resulted from increased glucose uptake into organ tissues, or reduced release of glucose from the liver. A study using stable isotopes to trace individual tissue uptake of glucose in endotoxic steers treated with

and without gonadal steroids is needed to measure rates of glucose appearance in and disappearance from blood.

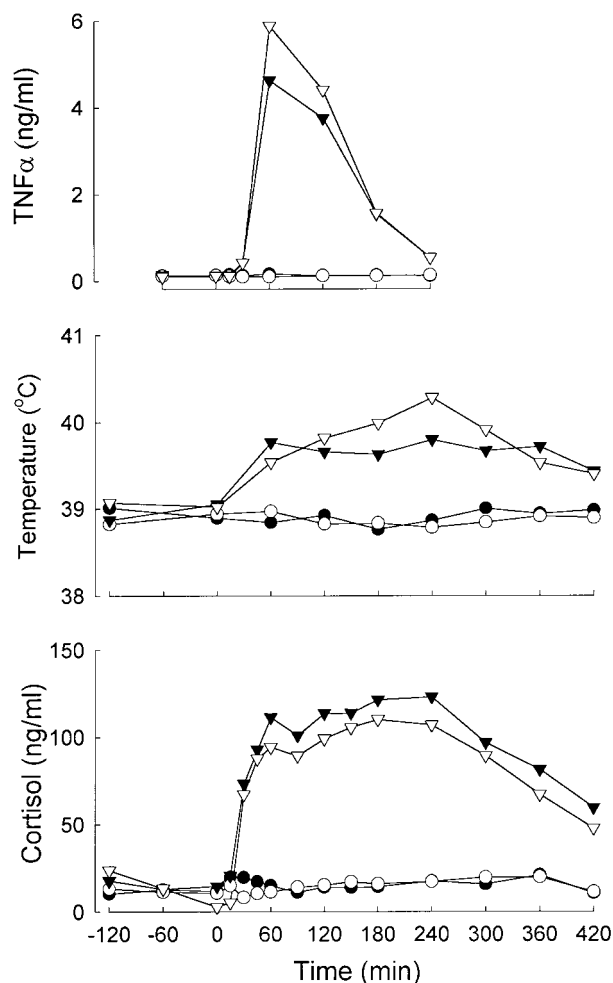
Hypoglycemia develops after initial hyperglycemia and results from increased uptake of glucose into diaphragm, spleen, liver and lung when hepatic glycogen stores are depleted and during a reduced rate of gluconeogenesis (Lang *et al.* 1985, 1987, 1993). Glucose tolerance was



**Figure 5** Least squares mean concentrations of IGF-I for 3 days after i.v. injection of vehicle or LPS (1 µg/kg BW) on day 0. Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Asterisks denote lower concentrations of IGF-I in LPS-treated groups compared with non-LPS-treated groups ( $*P < 0.05$ ). In addition, concentrations of IGF-I were higher in S/E than C/E on day 3 ( $^aP < 0.05$ ) but not different from C/C or S/C. Pooled S.E.M. = 10.6.



**Figure 6** Least squares mean concentrations of NEFA and BUN from 2 days before to 4 days after i.v. injection of vehicle or LPS (1 µg/kg BW) on day 0. Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Concentrations of NEFA were significantly different ( $*P < 0.05$ ) between S/E and C/E group means 12 h after injection of LPS. In addition, concentrations of BUN were lower in steroid-treated than non-steroid-treated groups on all days ( $^aP < 0.1$ ;  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ ). Pooled S.E.M. were 0.03 for NEFA and 0.9 for BUN.



**Figure 7** Least squares mean concentrations of TNF $\alpha$  from 2 h before to 4 h (TNF $\alpha$ ) or 7 h (cortisol and body temperature) after i.v. injection of vehicle or LPS (1  $\mu$ g/kg BW). Treatments were: non-steroid-treated controls (C/C, ●); steroid-treated controls (S/C, ○); non-steroid treated and injected with LPS (C/E, ▼); and steroid treated and injected with LPS (S/E, ▽). Pooled s.e.m. were 0.91 for TNF $\alpha$ , 0.2 for body temperature and 7.88 for cortisol.

tested at 6 h during early hypoglycemia and at 24 h when steers were recovering from hypoglycemia. It was reasoned that S/E-treated steers would recover earlier than C/E-treated steers and, therefore, would have increased energy requirements. Therefore, if LPS did not affect steers implanted with E<sub>2</sub>/P<sub>4</sub> as severely as controls, glucose t<sub>1/2</sub> would be shorter at 6 and 24 h in S/E- compared with C/E-treated steers. However, glucose t<sub>1/2</sub> did not differ between LPS-treated groups, although it was shorter in LPS- than in non-LPS-treated groups. In addition, insulin AUC increased in LPS-compared with non-LPS-treated groups, which indicates insulin resistance. Moreover, insulin AUC was greater in S/E- than C/E-treated steers indicating greater insulin resistance in S/E-treated steers.

It is not clear why increased insulin resistance in S/E-treated steers was also associated with lessened severity of endotoxemia. However, uptake of glucose into muscle increases via non-insulin-mediated glucose uptake (NIMGU) pathways during sepsis because skeletal muscles become insulin resistant (Lang *et al.* 1990, Virkamäki & Yki-Järvinen 1994). Therefore, it is possible that E<sub>2</sub>/P<sub>4</sub> implants reduced both NIMGU and insulin-mediated glucose uptake in skeletal muscles, which would spare more glucose for neural, cardiovascular and macrophage-rich tissues during endotoxemia.

Release of insulin from the pancreas was delayed 60 min after onset of hyperglycemia in LPS-treated steers. Moreover, concentrations of insulin were reduced from 30 to 60 min in LPS-treated steers, which indicates that another factor is involved in regulating release of insulin. One candidate factor is somatostatin (SS), which is synthesized in neurons in the hypothalamus (Leshin *et al.* 1994) and in D-cells of the pancreas and gastrointestinal tract (McIntosh 1985, Schusdziarra 1996). In rats, injections of LPS stimulate release of SS, which is probably released from the pancreas (Yelich *et al.* 1993, Yelich & Witek-Janusek 1994). Those authors also observed that concentrations of insulin did not increase immediately after the onset of hyperglycemia. SS also inhibits release of insulin from  $\beta$ -cells (Johnson *et al.* 1975). Therefore, it is possible that reduced concentrations of insulin and delayed release of insulin in the present study were due to increased concentrations of SS released from the pancreas.

GH was released in a synchronized pulse between 30 and 90 min. This pulse occurred at the same time that concentrations of insulin were reduced and concentrations of glucose were elevated in plasma. It is well established that insulin and GH are negatively related (Bassett 1972, 1974), but it is not as well understood how, or if, they regulate the release of each other. In the current study, concentrations of insulin were inversely related to concentrations of GH from 0 to 360 min, which is consistent with insulin regulating concentrations of GH. In contrast, concentrations of glucose were not significantly related to concentrations of GH. However, both insulin-induced hypoglycemia and i.c.v. injection of 2-deoxyglucose reduce growth hormone-releasing hormone (GHRH)-induced release of GH in sheep (Sartin *et al.* 1988a). Moreover, using the glucose clamp technique, Murao *et al.* (1994) showed that changes in concentrations of glucose, either hyper- or hypoglycemia, increase SS mRNA in the hypothalamus of rats. Furthermore, injections of insulin did not affect concentrations of GH during euglycemia in that study. An alternative explanation is that increased concentrations of NEFA suppressed concentrations of GH. Infusion of NEFA suppresses GHRH-induced release of GH (Sartin *et al.* 1988a) and concentrations of NEFA were elevated 12 h after injection of LPS in the current study. However, concentrations of NEFA were not measured at frequent intervals from 0 to

4 h to enable an appraisal of their relationship to concentrations of GH. Therefore, it is suggested that a glucose-induced increase in synthesis of SS in the hypothalamus (Murao *et al.* 1994) leads to increased release of SS into hypophysial-portal vessels and inhibits release of GH from the anterior pituitary gland. However, the possibility that insulin plays a role in regulating release of GH cannot be excluded.

Pulsatile release of GH was assessed over the entire 8 h period, which included the 2 h period before and the 6 h period after injection of LPS. Separating the analysis into periods before and after injection of LPS was considered, but was not performed because it was reasoned that a 2 h period was insufficient for meaningful information on pulsatile release of GH. E<sub>2</sub>/P<sub>4</sub> implants increased mean concentrations of GH in S/E- and S/C-treated steers, despite an increased pulse of GH between 30 and 90 min and suppressed release from 120 to 180 min after i.v. injection of LPS. Pulse secretion parameters indicate that these increased means resulted from an increased pulse amplitude of GH because pulse frequency was not altered. This main treatment effect of E<sub>2</sub>/P<sub>4</sub> implants on pulse amplitude of GH in S/E- and S/C-treated steers is inconsistent with the marked differences in the pattern of concentrations of GH in plasma between LPS- and non-LPS-treated steers. For example, increased amplitude and mean concentrations can be attributed to the pulse between 30 and 90 min and increased concentrations of GH after 180 min. However, given that pulse secretion parameters were also similar between C/E- and C/C-treated steers when a similar difference existed between their pattern of concentrations of GH in plasma, it is likely that E<sub>2</sub>/P<sub>4</sub> implants increased GH amplitude. Breier *et al.* (1988) and Hayden *et al.* (1992) measured higher concentrations of GH in E<sub>2</sub>-treated cattle, which supports this finding.

Administration of GH and/or IGF-I reverses catabolic states and accelerates protein gain in subjects and animals on calorie-restricted diets and during sepsis (Clemmons *et al.* 1992, Jurasinski & Vary 1995, Zhao & Donovan 1995). Furthermore, concentrations of IGF-I are higher during growth (Suttie *et al.* 1989) and are reduced during periods of restricted nutrition and fasting (McGuire *et al.* 1992, Thissen *et al.* 1994) and during infection and disease (Elsasser *et al.* 1988, 1995). Therefore, it was reasoned that calves recovering at a faster rate would have higher concentrations of IGF-I in their plasma. In the current study, concentrations of IGF-I were significantly higher in S/E- than C/E-treated steers and not significantly different between S/E- and C/C-treated steers on day 3, which suggests that S/E-treated steers were recovering faster from endotoxemia than C/E-treated steers.

Lungs are particularly sensitive to LPS, and respiratory distress is a common feature of endotoxemia. Activated polymorphonuclear neutrophils migrate into alveoli and septa, adhere to endothelial cells and release oxygen

radicals which damage or destroy those endothelial cells (Welbourn & Young 1992, Domenici-Lombardo *et al.* 1995). This generalized lung injury is associated with tachycardia, hyperventilation, hypotension, bronchoconstriction, hypoxemia and reduced lung compliance. In addition, it was noticed that sheep and cattle cough, grunt and drool saliva after injection of LPS (our unpublished observations). The duration of panting, coughing or grunting and drooled saliva was monitored for a behavioral index of steers' wellbeing. The present data show that E<sub>2</sub>/P<sub>4</sub> implants abolish drooling of saliva, reduce duration of coughing and grunting, but do not alter duration of panting. It is speculated that lung injury is reduced in steers implanted with E<sub>2</sub>/P<sub>4</sub>. An important function of saliva is to buffer the rumen against changes in pH (Kay 1960). We suggest that increased secretion of saliva may reflect a mechanism to buffer the rumen against infection-induced changes in pH, which has been shown to occur during induced disease (Sager *et al.* 1990). Therefore, E<sub>2</sub>/P<sub>4</sub> implants may suppress LPS-induced changes to the gastrointestinal tract.

An important observation in this study was that E<sub>2</sub>/P<sub>4</sub> implants did not alter concentrations of cortisol, or reduce concentrations of TNF $\alpha$  in S/E- compared with C/E-treated steers. This suggests that reduced severity and enhanced recovery from endotoxemia in S/E- compared with C/E-treated steers cannot be attributed to immunosuppressive and anti-inflammatory actions of glucocorticoids. Nor can it be attributed to reduced concentrations of TNF $\alpha$  and/or reduced availability of cytokine receptors. Gonadectomized male and female mice have enhanced release of adrenocorticotropin, corticosterone and TNF $\alpha$  after injection of LPS and replacement of gonadal steroids blocks these increases (Spinedi *et al.* 1992). Cortisol and potent analogs inhibit transcription of interleukin (IL)-1 $\beta$  (Lee *et al.* 1988). Moreover, cortisol blocks increased concentrations of TNF $\alpha$  and reduces concentrations of soluble TNF receptors and IL-1 receptor antagonist in a time-dependent manner in rats (Barber *et al.* 1993, 1995). In the present study, duration and degree of fever were not different between S/E- and C/E-treated steers. Therefore, E<sub>2</sub>/P<sub>4</sub> implants act via other mechanisms to reduce severity of endotoxemia in steers.

It is concluded that E<sub>2</sub>/P<sub>4</sub> implants reduce severity of endotoxemia in steers. The mechanism of action is not known, but it does not appear to act through reducing concentrations of TNF $\alpha$  or via anti-inflammatory and immunosuppressive actions associated with the hypothalamo-pituitary-adrenal axis.

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