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## Effect of endotoxin challenge on hepatic 5'-deiodinase activity in cattle

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### Abstract

Thyroid status is compromised in a variety of acute and chronic infections and toxin-mediated disease states. Conversion of thyroxine ( $T_4$ ) into the metabolically active hormone, triiodothyronine ( $T_3$ ), is catalyzed by 5'-deiodinase (5'D). Our objective was to determine the effect of endotoxin (LPS) challenge with and without L-arginine (Arg) infusion on hepatic activity of 5'D and plasma concentrations of  $T_4$  and  $T_3$ . In a  $2 \times 2$  factorial, beef heifers (275–310 kg b.wt.) were fed low (8% CP; 6.5 kg/d) or high (14% CP; 7.2 kg/d) isocaloric protein diets (1.96 Mcal/kg DM) for 10 d before LPS challenge. L-Arginine in saline (0.5 g/kg b.wt.) or saline alone was infused iv throughout an 8 hr period starting 2 hr before bolus LPS injection (*Escherichia coli*, 055 : B5; 0.2  $\mu\text{g}/\text{kg}$ ; iv). Blood samples were collected at -2, 0, 3, 6, 12, and 24 hr relative to LPS injection. Liver samples were obtained 20 hr before, and then 6 and 24 hr after LPS challenge using a biopsy needle. Plasma  $T_4$  and  $T_3$  concentrations were not affected by dietary CP or Arg. Compared with levels at 0 hr, LPS challenge decreased plasma  $T_4$  ( $P < 0.01$ ) and  $T_3$  ( $P < 0.001$ ), respectively, 8.4% and 28.9% at 6 hr and 19.7% and 31.3% at 24 hr. Consistent with these changes, the  $T_3 : T_4$  ratio was lower than that at 0 hr ( $P < 0.001$ ) 22.0% at 6 hr and 13.5% at 24 hr. Hepatic 5'D activities 20 hr before LPS injection were  $2.80 \pm 0.11 \text{ nmol } \text{I}^- \cdot \text{hr}^{-1} \cdot \text{mg protein}^{-1}$  and decreased 24 hr after LPS, respectively, 45.4% ( $P < 0.01$ ) and 17.6% ( $P < 0.05$ ) in saline- and Arg-infused heifers. The results indicate that mild LPS challenge in cattle inhibits hepatic generation of  $T_3$  and decreases plasma concentrations of thyroid hormones. The data also suggest that the impact of LPS on 5'D activity in liver can be altered by Arg supplementation. © 2000 Elsevier Science Inc. All rights reserved.

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## 1. Introduction

Thyroid status of growing animals is an important regulator of metabolic rate [1] and affects the amount of nutrients used for maintenance and growth [2]. Thyroid hormones may impart differences in sensitivity of various organs to other regulatory hormones as well as directly influence metabolic rate of individual organs. Thyroxine ( $T_4$ ), the predominant thyroid hormone in the circulation, has little, if any, inherent biologic activity [3]. Whereas  $T_4$  is synthesized only in the thyroid, the most metabolically active thyroid hormone, triiodothyronine ( $T_3$ ), is produced by enzymatic 5'-deiodination of the  $T_4$  within the thyroid gland and in extrathyroidal tissues [4,5]. Because  $T_3$  is a very potent regulator of energy [6] and protein metabolism [7], the extrathyroidal activity of iodothyronine 5'-deiodinase (5'D) is an important control point for regulating the metabolic status of animal tissue in various physiological and pathologic situations [3,8].

A number of infectious and inflammatory illnesses are associated with profound changes in thyroid status in humans [3,9] and in laboratory [10] and domestic [11,12] animals. This so-called euthyroid sick syndrome (or low  $T_3$  syndrome) observed during systemic non-thyroidal illness (NTI) includes a decrease in serum concentration of  $T_3$ , an increase in serum reverse- $T_3$  ( $rT_3$ ) and, in severe cases, a decrease in  $T_4$  and thyrotropin (TSH) concentrations. Most of these changes are caused by a lower  $T_3$  production rate and a decreased  $rT_3$  metabolic clearance rate due to diminished extrathyroidal 5'D activity [3,9]. It has been reported that some of the cytokines (e.g., tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ] and interleukin-1 [IL-1]) produced by the activated immune system during systemic NTI are important mediators of changes in thyroid status including inhibition of TSH release from pituitary cells [13,14] and decreased activity of type-I 5'D in thyroid [15] and liver tissue [14,16].

Endotoxin (LPS), a model effector of the acute phase response (APR) of bacterial infection and a strong stimulus for cytokine release [17,18,19], has been shown to decrease circulating concentrations of  $T_3$  in dogs [20] and  $T_3$  and  $T_4$  in humans [21], rodents [18], cattle [22,23], and goats [12,24]. Furthermore, LPS administration in mice decreased the expression of liver type I 5'D mRNA [18,25].

The purpose of the present study was to investigate in cattle the effect of LPS challenge with and without L-arginine (Arg) infusion on hepatic 5'D activity and plasma concentrations of  $T_4$  and  $T_3$ . Arginine supplementation has been reported previously to improve the response to disease stress in experimental animals [26]. Also, Arg infusion induces the release of growth hormone (GH) and insulin [26], the hormones shown to stimulate extrathyroidal activity of 5'D [8].

## 2. Materials and methods

### 2.1. Animals and experimental design

This experiment was performed in accordance with approval of the Animal Care and Use Committee at the USDA Agricultural Research Service (Beltsville, MD, USA). The experiment was conducted as an unbalanced  $2 \times 2$  factorial in two replications with protein level

and Arg infusion as main treatments. Two different protein diets were used because this experiment was a part of a larger study that examined the nutritional regulation of nitric oxide (NO) and TNF- $\alpha$  responses to LPS challenge in cattle [27]. In the first replication, eight crossbred heifers (275–310 kg b.wt.) were used with two animals assigned to each treatment. In the second replication, the same heifers were used and two additional heifers were added. The heifers were fed low (LP: 8% CP; 6.5 kg/d per heifer) or high (HP: 14% CP; 7.2 kg/d per heifer) isocaloric protein diets (1.96 Mcal ME/kg DM) for 10 d before LPS administration. Either Arg (0.5 g/kg b.wt.) in saline or saline alone was infused via a jugular cannula for 8 hr starting at 6:00 a.m. with 1/3 of total Arg infused before LPS administration (*E. coli*, 055: B5; 0.2  $\mu$ g/kg; iv) at 8:00 a.m. Treatment assignments for heifers were switched between the first and second replication such that heifers on HP became LP, heifers on LP became HP; additionally, the assignment to Arg or saline infusion was reversed. The two extra heifers in replication two were assigned to LP + saline and HP + Arg. At least 3 wk elapsed between LPS challenges in animals used in both replicates. Three additional heifers on the LP diet served as controls and were not challenged with LPS nor infused with Arg. Blood samples were obtained at -2, 0, 3, 6, 12, and 24 hr relative to LPS injection. Liver biopsy samples were collected 20 hr before, and then 6 and 24 hr after LPS injection using a commercial biopsy instrument (True-Cut; Baxter, Columbia, MD, USA) as previously described [27]. Biopsy samples were immediately frozen in liquid nitrogen. Blood plasma samples were stored at -20°C and biopsy samples at -80°C until assayed.

## 2.2. Hormone determination

Plasma  $T_4$  and  $T_3$  concentrations were determined in duplicate by using RIA kits (ICN Biomedicals, Inc., Carson, CA, USA) validated for bovine plasma [28]. For both hormones, intra-assay and inter-assay coefficients of variation were less than 6%.

## 2.3. 5'D determination (type-I)

Outer-ring deiodinating activity (5'D) was determined by quantifying the  $^{125}\text{I}^-$  released from 3,3',5'-[ $^{125}\text{I}$ ]- $T_3$  (r $T_3$ ) as previously described [28]. In brief, tissue samples of liver were homogenized in 0.01 M HEPES buffer (pH 7.0, 0.25 M sucrose, 5 mM EDTA) by using a Polytron homogenizer (Brinkman Instruments Inc., Westbury, NY, USA). After centrifugation (30 min at 2000  $\times$  g), the supernatant was incubated for 5 min in 0.1 M phosphate buffer (pH 7.0, 1 mM EDTA) in the presence of 5 mM dithiothreitol (DTT) at 37°C with approximately 80,000 c.p.m. of [ $^{125}\text{I}$ ]-r $T_3$  (DuPont–New England Nuclear, Boston, MA, USA) and 500 nM of unlabeled r $T_3$  (Calbiochem, La Jolla, CA, USA). The released  $^{125}\text{I}^-$  was isolated as trichloroacetic acid (TCA)-soluble radioactivity. The 5'D activity was expressed as nmol  $\text{I}^- \cdot \text{hr}^{-1} \text{mg protein}^{-1}$ . Protein concentration in homogenates was determined with bicinchoninic acid reagent and BSA as a standard (Pierce Chemical Co., Rockford, IL, USA).

#### 2.4. Statistical analysis

Data are presented as mean  $\pm$  SE. Data were initially analyzed by the GLM procedure of SAS [29] by using a  $2 \times 2$  factorial model split-plot-in-time with diet, Arg infusion and their interaction as main plot effects and time as the subplot. When a significant *F*-test result was found ( $P < 0.05$ ), the least significant difference was used to separate appropriate group means. Because protein level of the diets (HP versus LP) did not affect any of the endpoints studied, the data presented in the figures were averaged across the protein level for each time after LPS challenge and Arg or saline infusion.

### 3. Results

Administration of LPS to heifers resulted in transient signs of systemic illness characterized by an increase in rectal temperature for 3 to 4 hr ( $1.1 \pm 0.1^\circ\text{C}$  increment from base temperature at 0 hr to peak temperature at 3 hr,  $P < 0.001$ ), a short period of labored breathing, slight coughing that dissipated within 3 hr after LPS, mild diarrhea and lethargy that was not apparent 8 hr after LPS.

Changes in plasma concentration of  $T_4$  and  $T_3$  after LPS challenge are presented in Fig 1. In control heifers (CON) injected and infused with 0.9% saline instead of LPS and Arg, respectively, plasma concentrations of  $T_4$  and  $T_3$  and plasma  $T_3 : T_4$  molar ratios were stable throughout the 24 hr. After LPS challenge no differences ( $P > 0.05$ ) were observed in plasma profiles of  $T_4$ ,  $T_3$  and  $T_3 : T_4$  ratio between heifers infused with Arg (+Arg) or saline (–Arg). Compared with the average concentrations at zero time (combined –Arg and +Arg group:  $T_4$ ,  $75.5 \pm 1.6$  ng/ml;  $T_3$ ,  $1.92 \pm 0.05$  ng/ml), single LPS injection decreased plasma  $T_4$  and  $T_3$ , respectively, 8.4% ( $P < 0.05$ ) and 28.9% ( $P < 0.01$ ) at 6 hr, 33.9% and 53.8% ( $P < 0.01$ ) at 12 hr, and 19.7% and 31.3% ( $P < 0.01$ ) at 24 hr. Therefore, the plasma  $T_3 : T_4$  ratios after LPS challenge decreased ( $P < 0.001$ ), as compared to the average value at zero time ( $0.031 \pm 0.001$ ), 22.0, 29.0, and 13.5%, respectively, at 6, 12, and 24 hr (Fig. 1, bottom panel). During the first 3 hr after LPS administration no changes in plasma concentration of thyroid hormones were noted.

In all experimental heifers no changes in hepatic 5'D activities (Fig. 2) were observed 6 hr after LPS challenge. Compared with pre-challenge values (–20 hr:  $2.80 \pm 0.11$  nmol  $\text{I}^- \cdot \text{hr}^{-1} \cdot \text{mg protein}^{-1}$ ), hepatic 5'D activities 24 hr after LPS injection decreased 45.4% ( $P < 0.01$ ) in saline-infused heifers but only 17.6% ( $P < 0.05$ ) in Arg-infused heifers (time  $\times$  Arg interaction,  $P = 0.06$ ). Therefore, at 24 h, the 5'D activities were lower ( $P < 0.01$ ) in saline- than in Arg-infused animals.

### 4. Discussion

The experiment described herein was a replicated design. It is well documented that repeated exposure to LPS challenge results in the development of tolerance as previously described for cattle with two LPS challenges administered 5 d apart [17]. However, in this experiment there was no evidence of tolerance. Parameters of thyroid status responded to LPS challenge identically in the two experimental replications, separated by a 3-wk interval.

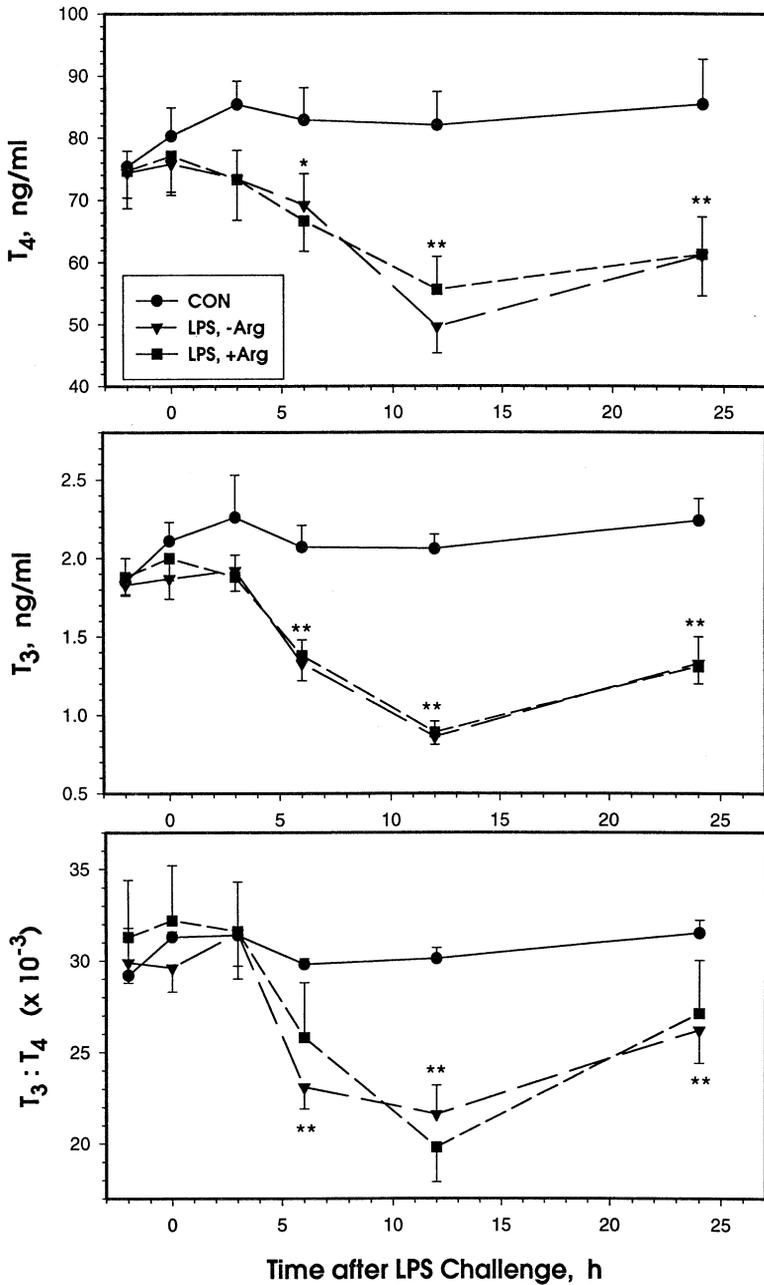


Fig. 1. Plasma concentration of thyroxine (T<sub>4</sub>, upper panel), triiodothyronine (T<sub>3</sub>, middle panel), and T<sub>3</sub>:T<sub>4</sub> molar ratio (bottom panel) after endotoxin challenge (LPS: 0.2 μg/kg, iv bolus injection at time 0) in heifers infused with L-arginine (+Arg, 0.5 g/kg) or 0.9% saline (-Arg). Control heifers (CON) were not injected with LPS or infused with Arg. Values represent means ± SE of 9 (-Arg and +Arg) or 3 (CON) heifers/group. Because infusion of Arg did not affect the response to LPS challenge in any parameter, the asterisks indicate the effect of LPS for data averaged over combined -Arg and +Arg group for each time (\*P < 0.05, \*\*P < 0.01 versus time 0).

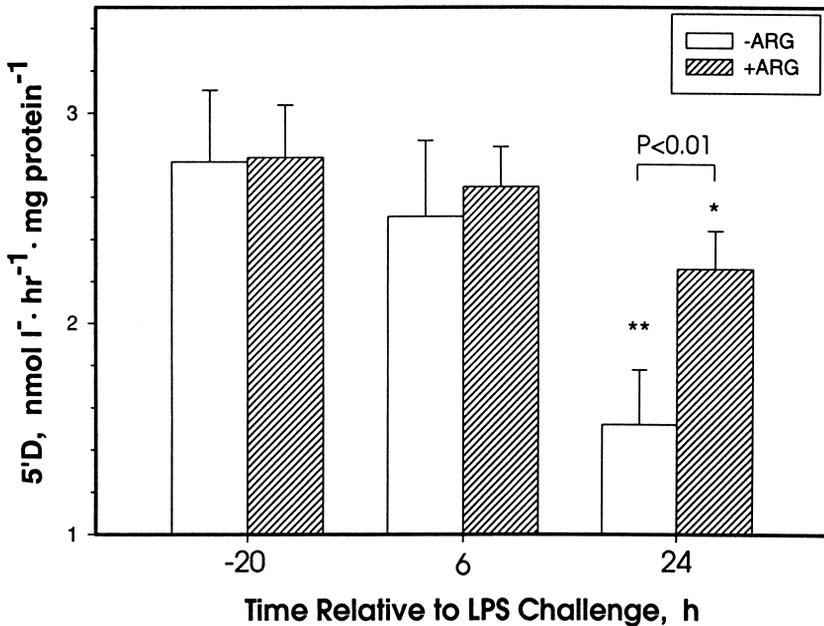


Fig. 2. Hepatic activity of type I 5'-deiodinase (5'D) after endotoxin challenge (LPS: 0.2  $\mu\text{g}/\text{kg}$ , iv bolus injection at time 0) in heifers infused with L-arginine (+Arg, 0.5 g/kg) or 0.9% saline (-Arg). Values represent means  $\pm$  SE (\* $P < 0.05$ , \*\* $P < 0.01$  versus time -20 hr in respective treatment group;  $n = 4\text{--}5$  heifers/treatment).

Also, as reported previously [27], response of other parameters to LPS challenge in these same heifers (plasma increases in TNF- $\alpha$  and nitrate + nitrite concentrations) did not differ between replications. Thus, in this replicated experiment, the host responses to the second LPS challenge were not blunted due to prior exposure to LPS and tolerance was not a confounding factor in the design.

The data presented here show that bolus administration of a low dose of LPS to cattle significantly reduced circulating concentrations of plasma  $T_4$  and  $T_3$ . The time course of these changes, and the decline in plasma  $T_3 : T_4$  ratio, are in agreement with changes previously observed after single injection of LPS in humans [21], mice [18], goats [24], and lactating cows [22]. In the present experiment, we chose a dose of LPS [17] that caused transient signs of response (e.g., increased rectal temperature for 3 to 4 hr, a short period of labored breathing and coughing, decreased plasma glucose, and increased plasma cortisol and TNF- $\alpha$  for 4 hr) without prolonged and severe response or mortality. Despite mild symptoms of endotoxemia that disappeared within 8 hr, plasma  $T_3$  and  $T_4$  concentrations were still significantly suppressed 24 hr after LPS injection. Similar prolonged responses to low single doses of LPS were reported in plasma  $T_3$  and  $T_4$  in goats [24] and in serum  $T_3$  in calves [23] suggesting that in ruminants, thyroid status may be very sensitive to bacterial infection or toxin challenge.

The overall decrease in plasma concentration of  $T_4$  and  $T_3$  accompanied by decreased  $T_3 : T_4$  ratio indicates that LPS administration could 1) down-regulate pituitary TSH secretion, 2) diminish thyroid sensitivity to TSH stimulation, 3) suppress synthesis and secretion

of thyroid hormones, and/or 4) decrease extrathyroidal 5'-deiodinating activity resulting in reduced  $T_3$  generation. In the present experiment we did not measure TSH concentration in heifers and, to our knowledge, there are no available published data on the effect of LPS on TSH in cattle. However, in our preliminary dose-response trial with crossbred steers and heifers (unpublished data), single challenge with LPS doses of 0.2, 1.0, and 3.0  $\mu\text{g}/\text{kg}$  b.wt. did not decrease plasma TSH concentrations over a 24 hr sampling period. These observations suggest that the early depression in circulating concentration of  $T_4$ ,  $T_3$ , and plasma  $T_3 : T_4$  ratio after LPS challenge in the present experiment was not related to reduced pituitary secretion of TSH. Nevertheless, the pattern of LPS-induced alterations in thyroid status could vary among species. Endotoxin administration to humans [21] and rats [30,31] decreased plasma concentrations of TSH,  $T_4$  and  $T_3$ . In mice, decreased plasma  $T_4$  and  $T_3$  after LPS injection was not accompanied by any changes in plasma TSH [18]. In dogs, LPS challenge decreased basal concentration of serum  $T_3$  but not  $T_4$  [20]. Treatment with LPS was also shown to decrease TSH-stimulated thyroidal secretion of  $T_4$  in rats [31] and to reduce peak serum concentration of  $T_4$  in dogs after TSH or TRH challenge [20]. Further studies will be required to determine whether any changes in TSH status are involved in the decreased thyroid activity after LPS administration in cattle.

In the present study, we demonstrated that administration of a low-level bolus of LPS to heifers decreased hepatic 5'D activity, suggesting that LPS may impair the extrathyroidal  $T_4$  to  $T_3$  conversion responsible for most of the circulating  $T_3$  in mammals [4]. However, decreased 5'D activities observed 24 hr but not 6 hr after LPS challenge (Fig. 2) suggest that the early reduction (6 to 12 hr) in plasma concentrations of thyroid hormones and  $T_3 : T_4$  ratio is related to reduced thyroid gland activity rather than to reduced extrathyroidal 5'D in liver. It has been suggested, based on indirect observations of changes in thyroid hormone concentrations in humans [21], mice [18], and dogs [20], that decreased extrathyroidal activity of type I 5'D plays an important role in lowering the concentration of circulating  $T_3$  during the acute phase of response to LPS administration. However, this hypothesis is supported only by a limited number of direct observations. Activity of 5'D in rat liver was not affected 15 and 24 hr after LPS administration [32]. In mice, a single sub-lethal dose of LPS decreased liver 5'D mRNA from 4 hr onwards, followed by decreased serum  $T_3$  at 8 hr and serum  $T_4$  at 24 hr [18,25]. This suggests that the decrease in thyroid hormones after LPS in mice is due to a two-phase response, an early inhibition of hepatic 5'D activity and a later inhibition of thyroid gland activity. In contrast, the present data suggest that, in cattle, reduced activity of hepatic 5'D 24 hr after LPS injection is a secondary event that is related to decreased concentration of plasma  $T_4$  and  $T_3$ , although a direct inhibitory effect of LPS on 5'D activity cannot be ruled out. Type I 5'D is directly regulated by circulating concentration of thyroid hormones,  $T_3$  in particular [8]. Thus, the decreased concentrations of plasma  $T_4$  and  $T_3$  due to an early depression of thyroid gland activity could induce the later inhibition of hepatic 5'D activity.

Mechanisms by which LPS affect the thyroid status are not completely understood. However, the cytokines, especially TNF- $\alpha$ , IL-1, IL-6 (IL-6), and interferon- $\gamma$  (INF- $\gamma$ ), have been implicated in the inhibition of thyroid hormone production [33]. Administration of LPS results in the release of these cytokines in rodents [18] and ruminants [17,19,34]. As reviewed recently by Bartalena et al. [33], all steps of thyroid hormone synthesis, secretion

and peripheral metabolism may be negatively affected by cytokines. In the heifers used in the present experiment, plasma concentrations of immunoreactive TNF- $\alpha$  peaked 1 hr after LPS administration and returned to the baseline by 4 hr [27]. Although there are no available data on the effect of TNF- $\alpha$  (or any other cytokine) on thyroid status of cattle, TNF- $\alpha$  administration to mice and rats decreases serum concentrations of T<sub>3</sub>, T<sub>4</sub>, and TSH, and decreases iodine uptake, and TSH-induced T<sub>3</sub> and T<sub>4</sub> release [16,35]. Also, in human thyrocytes in vitro, TNF- $\alpha$  inhibited basal and TSH-stimulated iodine uptake and iodothyronine release [36]. However, in contrast to these inhibitory effects of exogenous TNF- $\alpha$  on thyroid activity, neutralization of endogenous TNF- $\alpha$  did not prevent LPS-induced thyroidal suppression in mice [37] and humans [38]. These findings suggest that TNF- $\alpha$  alone may not play an important role in the alteration of thyroidal function after LPS challenge.

Conflicting results were reported concerning effects of TNF- $\alpha$  on extrathyroidal 5'D activity. Prolonged administration (3 to 5 d) of TNF- $\alpha$  to mice [35] and rats [39] did not influence 5'D activity in liver nor did acute administration of TNF- $\alpha$  to mice alter hepatic 5'D mRNA content [18]. However, in one study, 5'D activity in rat liver decreased 8 hr after TNF- $\alpha$  treatment, but increased after 3 d of repeated injections [16]. In vitro, stimulation of 5'D transcripts and 5'D activity by TNF- $\alpha$  was also reported for  $\Phi_1$  rat liver cells [40]. On the other hand, in vitro studies using rat thyroidal cell line FRTL-5 [15,41] have shown decreased concentration of mRNA and 5'D activity in response to treatment with TNF- $\alpha$  and other cytokines (IL-1, IL-6, INF- $\gamma$ ) that are usually released after an in vivo LPS challenge. The decrease in thyroidal generation of T<sub>3</sub> from T<sub>4</sub> may result in a decreased T<sub>3</sub> : T<sub>4</sub> ratio in thyroidal secretion and, as a consequence, in plasma [5]. These data from different animal species are consistent with our suggestion that in cattle, LPS challenge affects thyroidal activity before decreasing extrathyroidal T<sub>3</sub> generation. This early reduction in thyroidal activity, including reduced intrathyroidal T<sub>4</sub> to T<sub>3</sub> conversion (suggested by decreased plasma T<sub>3</sub> : T<sub>4</sub> ratio without changes in hepatic 5'D activity), may be mediated by increased concentrations of TNF- $\alpha$  and other cytokines released after LPS administration.

In the present study, Arg infusion during the APR restored the reduced activity of 5'D in liver 24 hr after LPS challenge, although it did not facilitate an increase in plasma concentrations of T<sub>3</sub> and plasma T<sub>3</sub> : T<sub>4</sub> ratio. These observations support our previous suggestion that in cattle, decreased 5'D activity in liver 24 hr after LPS administration was a secondary event related to reduced concentration of circulating thyroidal hormone. Furthermore, these data also indicate that alleviated extrathyroidal 5'D activity during disease stress in cattle may not be sufficient alone to compensate for the depressed thyroidal activity. Although in euthyroid mammals type-I 5'D in extrathyroidal tissues (e.g., liver) is responsible for most of the circulating T<sub>3</sub> [4], the contribution of this type of enzyme to overall T<sub>3</sub> production in hypothyroidism could be substantially decreased as shown in humans during NTI [42] and rats on selenium deficient diet [5] or propylthiouracil treatment [8]. Those observations indicate that most of the T<sub>3</sub> produced from T<sub>4</sub> during hypothyroidism could be generated by type-I 5'D in the thyroidal gland [5] or type-II 5'D in peripheral tissues [8]. In the present experiment, LPS administration to heifers induced hypothyroid state with plasma T<sub>4</sub> concentration significantly lower than in control animals. In addition, decreased transport of T<sub>4</sub> across plasma membrane into the hepatocytes, reported during NTI and fasting [42], could further reduce substrate availability for the type-I 5'D in liver. Therefore, alleviated hepatic

5'D activity in Arg-infused heifers in this experiment could not be efficient enough to increase overall production of  $T_3$  and plasma  $T_3:T_4$  ratio in the presence of reduced availability of  $T_4$  after LPS challenge.

We used the infusion of Arg to enhance substrate availability for urea cycle arginase and for nitric oxide synthase, and because of its purported claims to improve immune function and recovery from injury and disease stress [26,43]. This is the first report indicating that Arg may also protect the activity of 5'D in liver despite a general hypothyroid status observed during NTI. The underlying mechanism and physiological significance for this endocrine effect of supra-physiological doses of Arg is unclear. When rapidly infused iv, Arg has been shown to stimulate the release of several hormones including GH and insulin [26,43]. Both, GH in cattle [44] and insulin in rodents [8] increased 5'D activity in extrathyroidal tissues. Recently, we also demonstrated that short-term (3 to 5 d) administration of recombinant bovine GH to cattle blunted the magnitude of the release of TNF- $\alpha$  into the circulation and decreased the severity of some physiological responses to endotoxemia [17]. However, in the present experiment the rate of Arg infusion (0.5 g/kg in 8 hr) was much slower than that needed to affect GH secretion (0.5 g/kg in 10 min). Mean plasma concentrations of GH throughout the 8 hr of Arg infusion in the present experiment were not affected by Arg infusion or LPS administration (data not shown). It seems unlikely, therefore, that GH release by Arg was a factor in the observed effects of Arg on the 5'D response to LPS. Further studies are required to elucidate the possible role of other hormonal factors or direct involvement of Arg in the protection of 5'D activity during LPS challenge.

Finally, it is important to realize that complex sets of secretion, distribution and clearance kinetics are induced with the administration of LPS that can have major effects on measurable concentrations of hormones and metabolites in plasma. Cytokine and nitric oxide-mediated changes in vascular permeability, differential tissue and organ blood flow patterns, gene induction and repression, tissue oxygenation, mitochondrial function, transmembrane passage of substrates, and hormone signal transduction pathways interact at the cellular level to shape the temporal patterns of hormones in blood [45]. Although this issue is largely beyond the scope of the present study, the data presented here add a new piece to the puzzle by delineating the direct effect of LPS on the deiodinase aspect of thyroid metabolism.

In conclusion, single low-dose LPS administration in cattle decreases circulating concentrations of thyroid hormone followed by decreased activity of type I 5'D in liver. The time course of these changes indicates that decreased extrathyroidal  $T_3$  generation is a secondary event caused by reduced thyroid activity. Although Arg infusion alleviates 5'D activity in liver, it does not compensate for the depressed secretion of  $T_3$  from the thyroid gland. It could also be speculated that all changes in thyroid status after LPS administration were caused, directly or indirectly, by the surge in plasma concentration of TNF- $\alpha$ .

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Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

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