Technical note: Bovine acute-phase response after corticotrophin-release hormone challenge

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Technical note: Bovine acute-phase response after corticotrophin-release hormone challenge[1]

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ABSTRACT: The objective of this study was to evaluate plasma concentrations of cortisol, acute-phase proteins, and proinflammatory cytokines in beef steers after an intravenous corticotrophin-release hormone (CRH) infusion. Six weaned, halter-trained Angus steers (BW = 163 ± 7.0 kg; age = 203 ± 5.8 d) were fitted with indwelling jugular catheters on d −1 of the study and assigned to receive intravenously 0.1 μg of bovine CRH/kg of BW on d 0 of the study. Blood samples were collected every hour via jugular catheters from −1 to 8 h, and every 6 h via jugular venipuncture from 12 to 72 h relative to CRH infusion (0 h). Steer rectal temperature was assessed concurrently with each blood collection. Samples collected from −1 to 8 h relative to CRH infusion were analyzed for plasma concentrations of IL-1β and IL-6, tumor necrosis factor-α, interferon-γ, cortisol, ceruloplasmin, and haptoglobin, whereas samples collected from 12 to 72 h were analyzed for plasma concentrations of ceruloplasmin and haptoglobin only. Plasma cortisol concentrations were greater ($P < 0.01$) at 1, 2, and 3 h compared with prechallenge values (2.8, 2.3, 13.9, 8.2, and 5.7 ng/mL for −1, 0, 1, 2, and 3 h, respectively; SEM = 0.6). Mean postchallenge IL-6 concentrations were greater ($P = 0.04$) compared with mean prechallenge values (1.49 vs. 1.34 log pg/mL; SEM = 0.05), whereas IL-6 concentrations at 6 h were greater ($P \leq 0.05$) compared with prechallenge values (1.36, 1.33, and 1.61 log pg/mL at −1, 0, and 6 h, respectively; SEM = 0.09). Rectal temperatures were greater ($P < 0.01$) at 2 and 8 h compared with prechallenge values (38.9, 39.0, 39.3, and 39.5°C at −1, 0, 2, and 8 h, respectively; SEM = 0.07). Plasma ceruloplasmin concentrations were greater ($P = 0.03$) at 54 h (29.6, 31.5, and 35.2 mg/dL at −1, 0, and 54 h, respectively; SEM = 1.7), whereas plasma haptoglobin concentrations were greater ($P < 0.05$) at 54, 66, and 72 h compared with prechallenge values (3.3, 2.8, 4.3, 4.6, and 4.1 absorbance at 450 nm × 100 at −1, 0, 54, 66, and 72 h, respectively; SEM = 0.24). In conclusion, intravenous CRH infusion at 0.1 μg/kg of BW increased circulating concentrations of cortisol and stimulated the acute-phase response in halter-trained beef steers.

Key words: acute-phase protein, acute-phase response, bovine, corticotrophin-release hormone, proinflammatory cytokine

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INTRODUCTION

The acute-phase response is an important component of the innate immune system, but can be detrimental to livestock performance (Carroll and Forsberg, 2007). In cattle, circulating concentrations of acute-phase proteins were negatively associated with ADG of beef steers and heifers (Qiu et al., 2007; Cooke et al., 2009a) and with reproductive performance of beef cows (Cooke et al., 2009b). Hence, management strategies that lessen the magnitude of the acute-phase response have been shown to benefit cattle productivity and overall efficiency of beef operations (Duff and Galyean, 2007).

The physiological mechanisms associated with the bovine acute-phase response are not completely understood. In cattle, circulating concentrations of acute-phase proteins were increased upon infections and diseases (Carroll and Forsberg, 2007). Recently, Carroll et al. (2009) characterized the bovine acute-phase response after a LPS challenge, which may serve as a research model to further study the acute-phase response after pathogenic stimuli. Excitable temperament and stressful procedures such as weaning and transportation have also been associated positively with concentrations of

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acute-phase proteins in cattle (Arthington et al., 2005; Cooke et al., 2009a). However, the mechanisms by which nonpathogenic stimuli trigger the acute-phase response are still unknown. Hepatic synthesis of acute-phase proteins were stimulated in vitro by addition of glucocorticoids to cultures of bovine liver slices (Higuchi et al., 1994). Therefore, we theorized that the neuroendocrine response to stress stimulates the bovine acute-phase response. The objective of this study was to determine whether increased plasma cortisol concentrations, achieved by corticotrophin-release hormone (**CRH**) infusion, would trigger the acute-phase response in cattle, serving as foundation for future studies and research models to investigate the effects of stress on the bovine innate immune system.

MATERIALS AND METHODS

The study was conducted in November 2009, and the animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee.

Animals

Six Angus steers were utilized in the study (d −1 to 3). All steers were weaned 70 d before the beginning of the study and exposed daily to halter-training techniques to become acclimated to human interaction; thus preventing confounding effects between human handling, weaning, and CRH challenge on the responses measured herein (Arthington et al., 2005; Curley et al., 2008; Cooke et al., 2009a). Beginning 5 d before and during the study, steers were housed in individual pens contained within an enclosed barn and offered free-choice water, mineral-vitamin mix, and meadow foxtail (*Alopecurus pratensis* L.) hay. From d −4 to −2, steers were weighed daily. Average BW and age at the beginning of the study were, respectively, 163 ± 7.0 kg and 203 ± 5.8 d. On d −1, all steers were fitted with an indwelling jugular vein catheter for serial blood collection according to the procedures described by Merrill et al. (2007). During the study, average daily temperature, maximum daily temperature, and minimum daily temperature were, respectively (°C), 4.7 ± 0.8, 14.5 ± 1.3, −5.2 ± 0.6.

Sample Collection and CRH Infusion

On d 0 of the study, all steers received 0.1 μg of bovine CRH/kg of BW (h 0 of the study). The proposed CRH dose was expected to increase plasma cortisol concentrations within biological values after challenge (Veissier et al., 1999; Curley et al., 2008) and simulate the increase in cortisol concentrations of cattle under stressful conditions (Crookshank et al., 1979; Arthington et al., 2003; Cooke et al., 2009a). Bovine CRH (#34-3-11, American Peptide Co. Inc., Sunnyvale, CA) was dissolved into physiological saline (0.9%) immediately before challenge and administered to steers via indwelling jugular catheters. Catheters were subsequently flushed with 10 mL of physiological saline to ensure that CRH reached the circulation, followed by 5 mL of heparinized saline (20 UI of heparin/mL of saline) to maintain catheter patency.

Blood samples (8 mL) were collected every hour via jugular catheters from −1 to 8 h, and every 6 h via jugular venipuncture (8 mL) from 12 to 72 h relative to CRH infusion (0 h). Immediately after each hourly collection, all catheters were flushed with 10 mL of physiological saline to replace fluid volume, followed by 5 mL of heparinized saline to maintain catheter patency. Catheters were removed after blood collection at 8 h. Concurrently with each blood collection, steer rectal temperature was assessed with a GLA M750 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA).

Blood Analysis

Blood samples that were collected via jugular catheters were immediately transferred into commercial blood collection tubes (10 mL, Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. All blood samples were placed immediately on ice and subsequently centrifuged at 2,500 × *g* for 30 min at 4°C for plasma collection. Plasma was frozen at −80°C on the same day of collection. Samples collected from −1 to 8 h relative to CRH infusion were analyzed for plasma concentrations of IL-1β and IL-6, tumor necrosis factor (**TNF**)-α, interferon (**IFN**)-γ, cortisol, ceruloplasmin, and haptoglobin. Samples collected from 12 to 72 h were analyzed for plasma concentrations of ceruloplasmin and haptoglobin only, given that plasma concentrations of these acute-phase proteins typically peak within 72 h after a stress stimulus (Arthington et al., 2005, 2008). Concentrations of cortisol were determined using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). Concentrations of ceruloplasmin and haptoglobin were determined according to procedures described previously (Demetriou et al., 1974; Makimura and Suzuki, 1982). Concentrations of IL-1β, IL-6, IFN-γ, and TNF-α were determined by a multiplex bovine-specific ELISA (SearchLight, Aushon Biosystems Inc., Billerica, MA). All samples were analyzed in duplicates. The intra- and interassay CV were, respectively, 2.9 and 6.2% for haptoglobin, 10.0 and 14.3% for ceruloplasmin, and 2.3 and 1.9% for cortisol. The intra- and interassay CV for cytokines was less than 15%.

Statistical Analysis

All data were initially tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Only cytokine data were

not normally distributed (W $\leq$ 0.69); therefore, data were log-transformed to achieve normality (W $\geq$ 0.90). All data were analyzed using the PROC MIXED procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Steer was considered the experimental unit. The model statement contained the effects of time (sampling hour or prechallenge vs. postchallenge values). Data were analyzed using steer as the random variable. The specified term for the repeated statement was time, and steer was included as subject. The covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least squares means and were separated by preplanned pairwise comparisons (PDIFF). For all analyses, significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

A time effect was detected ($P < 0.01$) for plasma concentrations of cortisol (Figure 1). Before the CRH challenge, plasma cortisol concentrations were reduced (2.84 and 2.31 ng/mL for −1 and 0 h, respectively), indicating that stress due to weaning, housing, human handling, and environmental factors such as extreme temperatures was minimal (Crookshank et al., 1979; Grandin, 1997; Silanikove, 2000). After the CRH challenge, plasma cortisol concentrations increased, reaching maximum values at 1 h and remaining greater ($P <$ 0.01) than prechallenge values until h 3 (2.8, 2.3, 13.9, 8.2, and 5.7 ng/mL for −1, 0, 1, 2, and 3 h, respectively; SEM = 0.6). Although a 5-fold increase in cortisol was detected when comparing prechallenge and peak value (Figure 1), the maximum concentration of cortisol detected herein was reduced compared with circulating concentrations of cortisol reported in stressed cattle (approximately 50 ng/mL; Crookshank et al., 1979; Arthington et al., 2003; Cooke et al., 2009a). Curley et al. (2008) reported that Brahman heifers with a calm temperament also experienced a 5-fold increase in plasma cortisol 1 h after intravenous infusion of 0.1 μg of CRH/kg of BW, although peak cortisol concentrations were approximately 50 ng/mL. In contrast, Holstein calves administered intravenously with 0.1 μg of CRH/kg of BW had similar prechallenge and peak cortisol concentrations compared with the present study (Veissier et al., 1999). Cattle with *Bos indicus* influence are more susceptible to stress of handling and consequently experience greater circulating cortisol concentrations compared with *Bos taurus* cohorts during blood collections (Hammond et al., 1996; Voisinet et al., 1997). Supporting this rationale, prechallenge concentrations of cortisol in calm *B. indicus* heifers reported by Curley et al. (2008) were approximately 10 ng/mL. In the present study, given that prechallenge concentrations of cortisol can be considered at baseline and homeostatic concentrations, the 5-fold increase in cortisol indicates that the CRH challenge activated the hypothalamus-pituitary-adrenal axis and mimicked a neuroendocrine response to stress (Veissier et al., 1999; Curley et al., 2008), although postchallenge plasma concentrations of cortisol were not as increased as in stressed cattle.

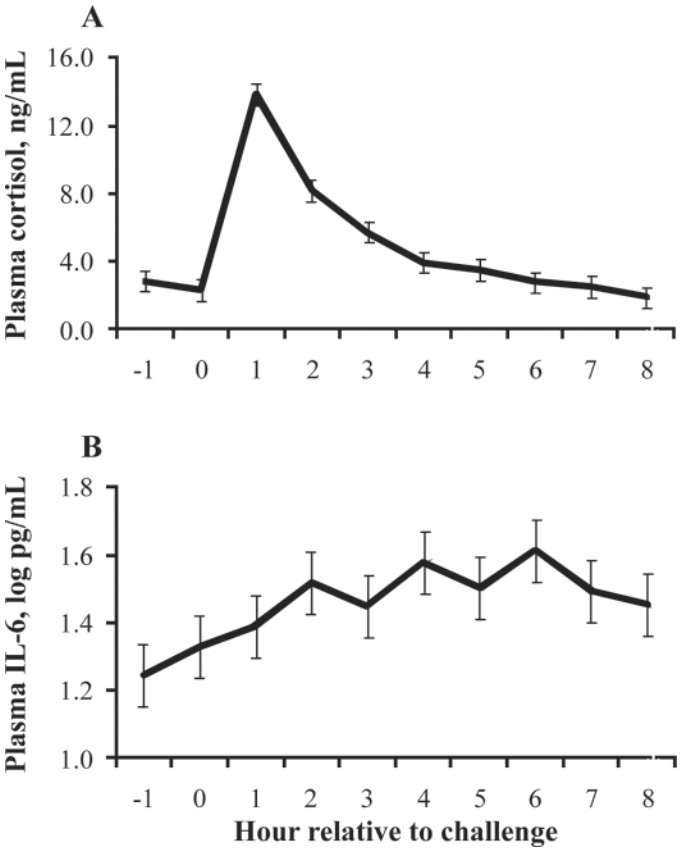

Figure 1. Plasma concentrations (±SEM) of cortisol (ng/mL; panel A) and IL-6 (log pg/mL; panel B) of steers receiving an intravenous corticotrophin-release hormone challenge (0.1 μg/kg of BW) immediately after blood collection at 0 h. A time effect was detected ($P <$ 0.01) for cortisol but not for IL-6 ($P = 0.27$). Compared with prechallenge values (h −1 and 0), cortisol concentrations were greater ($P <$ 0.01) at h 1, 2, and 3. However, mean postchallenge concentrations (h 1 to 8) of IL-6 were greater ($P = 0.04$) compared with prechallenge values (h −1 and 0), whereas IL-6 concentrations at 6 h relative to challenge were greater ($P \leq 0.05$) compared with prechallenge values.

No time effects were detected for plasma concentrations of the proinflammatory cytokines ($P \geq 0.27$; data not shown). However, mean postchallenge IL-6 concentrations (Figure 1) were greater ($P = 0.04$) compared with mean prechallenge values (1.49 vs. 1.34 log pg/mL, respectively; SEM = 0.05), whereas IL-6 concentrations at 6 h relative to challenge were greater ($P \leq$ 0.05) compared with prechallenge values (1.36, 1.33, and 1.61 log pg/mL at −1, 0, and 6 h, respectively; SEM = 0.09). This outcome indicates that the CRH infusion and consequent increased plasma cortisol concentrations triggered, at least partially, a proinflammatory cytokine response. Cortisol is known to directly reduce synthesis of proinflammatory cytokines by leucocytes (Kelley, 1988). However, acute increases in circulating cortisol, such as during a stress challenge, can indirectly stimulate an inflammatory response (Higuchi et al., 1994). One of the physiological effects of cortisol during

a stress response is to degrade body tissues, such as hepatic, adipose, and muscle cells (Nelson and Cox, 2005). This catabolic effect increases the amount of circulating nutrients available for the animal to cope with the stressor and restore homeostasis (Carroll and Forsberg, 2007). However, tissue degradation can be recognized by the innate immune system as a disruption in homeostasis (Abbas and Lichtman, 2007). In turn, leukocytes are activated and synthesize proinflammatory cytokines, predominantly IFN-γ, IL-1β, IL-6, and TNF-α (Gabay and Kushner, 1999) triggering the acute-phase and other immune responses to eliminate these damaged cells (Carroll and Forsberg, 2007). However, in the present study, only IL-6 responded to the CRH challenge, whereas the magnitude of this response can be considered moderate if compared with IL-6 increases after a LPS challenge (Carroll et al., 2009). Based on these results, greater CRH dosages and consequent cortisol concentrations might be necessary to stimulate substantial increases in plasma concentrations of IL-6 in addition to significant increases in plasma concentrations of IL-1β, TNF-α, and IFN-γ.

Upon synthesis and release into the circulation, proinflammatory cytokines elicit 2 major acute-phase responses: 1) increased body temperature by stimulating synthesis of PGE2, and 2) altered liver metabolism and gene regulation, favoring hepatic synthesis of the acute phase proteins (Carroll and Forsberg, 2007). In the present study, time effects were detected ($P < 0.01$) for rectal temperatures and plasma concentrations of the acute-phase proteins haptoglobin and ceruloplasmin (Figure 2), indicating that steers indeed experienced an acute-phase reaction. Immediately after CRH challenge, rectal temperatures increased, were greater ($P < 0.05$) than prechallenge values at 2 h and at 8 h (38.9, 39.0, 39.3, and 39.5°C at −1, 0, 2, and 8 h, respectively; SEM = 0.07), and returned to prechallenge baseline concentrations after 12 h. Rectal temperatures between 38.5 and 39°C are considered normal in young cattle under adequate environmental conditions (Merck, 1997). Herein, mean rectal temperatures were ≤39°C before and 12 h after CRH infusion, but >39°C from 1 h until 8 h relative to infusion (Figure 2). These results indicate that CRH challenge elicited a febrile

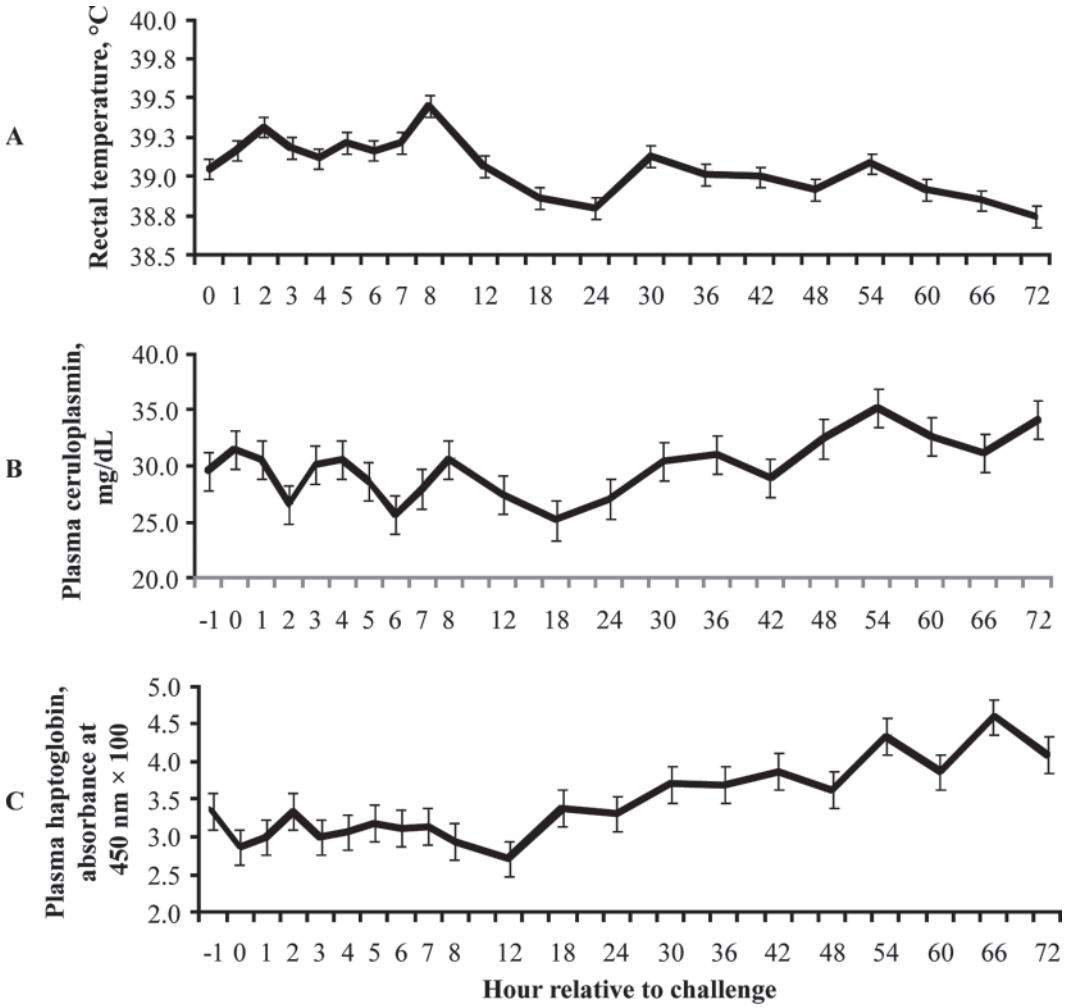

Figure 2. Rectal temperatures (°C; panel A; ±SEM) and plasma concentrations (±SEM) of ceruloplasmin (mg/dL; panel B) and haptoglobin (absorbance at 450 nm × 100; panel C) of steers receiving an intravenous corticotrophin-release hormone challenge (0.1 μg/kg of BW) immediately after sampling at 0 h. A time effect was detected ($P < 0.01$). Compared with prechallenge values (h −1 and 0), rectal temperatures were greater ($P < 0.01$) at 2 and 8 h, plasma ceruloplasmin was greater ($P < 0.05$) at 54 h, and plasma haptoglobin was greater ($P < 0.05$) at 54, 66, and 72 h.

response in steers, particularly at 2 and 8 h relative to challenge.

Compared with average prechallenge values (h −1 and 0; 30.6 mg/dL), plasma concentrations of ceruloplasmin were reduced ($P < 0.05$; SEM = 3.4) at 2 and 6 h (26.6 and 25.7 mg/dL, respectively), but greater ($P = 0.03$; SEM = 3.4) at 54 h (35.2 mg/dL). Plasma haptoglobin concentrations were greater ($P < 0.05$; SEM = 0.24) than average prechallenge values (3.35 absorbance at 450 nm × 100) at 42 h and after 54 h relative to CRH challenge (3.87, 4.33, 3.87, 4.60, and 4.10 absorbance at 450 nm × 100 for h 42, 54, 60, 66, and 72, respectively). The initial decrease in ceruloplasmin concentrations after CRH challenge can be attributed to an animal protective response during inflammatory processes to prevent endogenous Fe loss and deprive potential pathogens of this essential nutrient (Weinberg, 1984; Carroll et al., 2009). Increased plasma concentrations of haptoglobin and ceruloplasmin, particularly beginning at 54 h after the CRH challenge, can be associated with the IL-6 response detected herein, given that this proinflammatory cytokine is the major mediator for hepatic synthesis of acute-phase proteins (Gruys et al., 2005). Supporting this delayed increase in plasma concentrations of haptoglobin and ceruloplasmin, maximum concentrations of these proteins were only detected between 24 and 192 h after a stress challenge (Arthington et al., 2005, 2008).

According to these results, steers receiving an intravenous CRH challenge to increase plasma cortisol concentrations experienced an acute-phase response characterized by increased rectal temperatures and plasma concentrations of IL-6, haptoglobin, and ceruloplasmin. Greater plasma concentrations of acute-phase proteins after 54 h relative to CRH infusion may have been influenced, at least partially, by jugular catheterization and intensive blood collection via venipuncture. Indeed, tissue injury can elicit a local inflammatory response and contribute to circulating concentrations of acute-phase proteins (Baumann and Gauldie, 1994). However, temporary increases in plasma IL-6 and rectal temperatures within 8 h after CRH challenge indicate that increases in ceruloplasmin and haptoglobin were stimulated by an acute-phase inflammatory reaction due to CRH challenge. Nevertheless, an adequate control treatment, such as similar steers receiving an intravenous infusion of saline, was not included into the present study and would provide a more desirable assessment of the responses evaluated herein.

To our knowledge, this is the first manuscript reporting stimulatory effects of CRH challenge on proinflammatory cytokine and acute-phase response in cattle, which provides foundation for development of strategies that modulate the effects of stress on the bovine innate immune system. Furthermore, data reported herein indicate that additional research should be conducted to further characterize the bovine acute-phase reaction after nonpathogenic stress stimuli. These warranted research efforts should include an ideal control/placebo treatment group, differing CRH dosages, assessment of acute-phase proteins beyond 72 h relative to treatment, and evaluation of addition physiological mechanisms, such as leukocyte activity and circulating concentrations of eicosanoids.

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