

CHANGES IN SOMATOTROPIC AXIS RESPONSE AND BODY COMPOSITION DURING GROWTH HORMONE ADMINISTRATION IN PROGRESSIVE CACHECTIC PARASITISM

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A multistage protozoan parasitic disease was used as a cachexia model to study the effects of daily administration of bovine growth hormone (GH) on endocrine and body composition changes of young calves from the onset of the acute phase response (APR). Male calves averaging 127.5 ± 2.0 kg body weight were assigned to control, ad libitum fed, noninfected (C); ad libitum fed, infected (250,000 oocysts *Sarcocystis cruzi*, per os, I); noninfected, pair-fed (PF) to matched I-treatment calves and these respective same treatments in calves injected daily with GH (USDA-bGH-B1), 12.5 mg/calf/day, im) designated as C_{GH}, I_{GH} and PF_{GH}. GH injections were initiated on Day 20 postinfection (PI), 3 to 4 d before the onset of clinical signs of APR, and continued to Day 56 PI, at which time animals were euthanized for tissue collections. Abrupt increases in rectal temperature commensurate with up to 70% reduction in voluntary feed intake were observed in I and I_{GH} beginning 23–25 d PI. For the trial period between Days 20 and 56 PI, average daily carcass protein gains were 123, 52, 109, 124, 48, and 67 g/d and average daily carcass fat gains were 85, 11, 43, 71, –23, and 29 g/d for C, I, PF, C_{GH}, I_{GH}, and PF_{GH}, respectively. Effects of GH were significant for fat accretion and plasma urea depression. Rectus femoris was highly refractory to catabolic effects of infection while psoas major was significantly catabolized during infection. Plasma concentrations of insulin-like growth factor-I (IGF-I) increased significantly in all GH-treated calves between Day 20 and 23 PI. Plasma IGF-I declined well below Day 20 values in all infected calves from the onset of the APR through the end of the study. The decrease in plasma IGF-I concentrations in I and I_G was highly correlated with the magnitude of the fever response. Hepatic mRNA for GH receptor and IGF-I was decreased in infected calves. Hepatic microsomal membrane binding of ¹²⁵I-GH did not differ between groups. The data suggest that effects of GH and parasitism on tissue metabolism during disease may vary among different specific tissue pools. The data demonstrate that daily GH administration in young calves does not prevent lean tissue losses and may accelerate fat depletion associated with cachectic parasitism. Furthermore, the onset of APR overrode the capacity for GH to maintain elevated plasma concentrations of IGF-I, an effect not readily explained through changes in GH-receptor binding. © Elsevier Science Inc. 1998

INTRODUCTION

Body protein losses are apparent in a variety of physiologically stressful situations including malnutrition, sepsis and infection, and surgical trauma. In human clinical, as well as veterinary and agricultural, arenas reducing tissue wasting in association with disease stress remains a challenge. Tissue wasting associated with cachexia represents a

complex interaction of several converging influences encompassing anorexia, reduced efficiency of nutrient use for growth, increased caloric demand, reprioritized nutrient partitioning, and hypersecretion of inflammatory cytokines and glucocorticoids, all of which contribute to the predominant hypercatabolic state (1). The cachectic state may be associated with primary disease as in cancer but often is associated with secondary opportunistic manifestations of disease as occurs with parasitic infections accompanying immune deficiency or other states of compromised host defenses (2,3). A cachectic condition during the protracted acute phase response (APR) of calves infected with the protozoan parasite *Sarcocystis cruzi*, was characterized by chronic stunting, depletion of fat stores, and severe skeletal muscle wasting (4). Nutritional and endocrine components of the wasting syndrome of this parasitic disease were further defined (5,6) demonstrating that the reduced levels of nitrogen retention, rates of live weight gain and alterations in IGF-I status were much more severe than could be accounted for simply by nutritional deficit, a feature subsequently demonstrated for another cachectic state modeled with parasitism (7). Furthermore, membrane components of the developmental stage of *S. cruzi* that trigger APR after infection were shown to specifically stimulate macrophage-monocytes to release tumor necrosis factor- α (8), considered a major effector of cachexia. Kenison et al. (9) demonstrated that plasma concentrations of TNF- α of calves infected with *S. cruzi* were increased not at the onset of the acute phase response but rather beyond 42 d after oral inoculation, at a time when the cystic formations were developing in muscle and tissue wasting was prevalent. Kluger (10) stated that basal metabolic rate would need to increase as much as 30% to support a 1.5°C increase in body core temperature during fever. The combination of reduced nutritional intake plus increased caloric demand necessitates specific changes in muscle and adipose tissue metabolism (11). GH has been demonstrated to have beneficial effects in regard to both immune function (12) and slowing nitrogen loss during surgical trauma, burns, some septic conditions, and low nutritional intake (13,14). Moreover, estrogen-progesterone-based anabolic implants have been shown to decrease the severity and duration of diarrhea in cattle infected with *Eimeria* while reducing the magnitude of weight loss of infected calves (7). The effect of parasitic cachexia on changes in chemical composition of specific tissue beds and accompanying endocrine involvement is a relatively unexplored area of integrative physiology modeled in animals of significant economic importance. We used calf-sarcocystosis-pair feeding model (5) to explore the effects of GH to intervene in the progression of lean tissue loss and bolster the somatotrophic axis in terms of maintaining increased plasma concentrations of IGF-I.

MATERIALS AND METHODS

Animals and Inoculation/Infection Protocol. Thirty Holstein bull calves were obtained at 1 d of age after colostrum feeding and reared collectively in a parasite-free facility to the age of 3 mo. Initially, calves were fed whole milk at 10% body weight and weaned at 3 mo onto a solid feed with the development of rumination. The experimental diet consisted a pelleted feed assayed to provide 18.7% crude protein per kg dry diet (8.8% moisture) and 2.86 Mcal/kg (5) fed ad libitum and an additional 200 grams dry hay to facilitate digestion. The level of protein was chosen to ensure adequate nutrition to maintain any level of tissue gain response associated with GH administration (15). For the study, calves were inoculated orally at a body weight that allowed them to attain a predicted weight of 125 kg (approximately 4 mo old) at the onset of APR based on average daily rate of weight gain. Actual mean body weights of calves at the onset of APR was 127.2 ± 2.0 kg. Calves were maintained in individual pens to measure individual feed intakes and were fed ad libitum with water continuously available. Fresh feed was

provided once daily in the a.m.; feed intake was recorded; uneaten feed was discarded and adjustments to intake were made daily. Body weights were obtained weekly. Rectal temperatures were obtained daily at the same time (0800 h) each day. A parameter referred to as "response index" was calculated as the daily difference in rectal temperature of each infected calf from the mean daily temperature of control and paired calves measured between Day 24 and Day 38 relative to the timing of oral infection.

We selected an age of calf, approximately 4 mo old, which is normally rapidly growing but highly sensitive to infection stress, to assess the effects of GH on body composition and the somatotrophic axis during the APR period. Calves were assigned to one of six treatment groups: control (C, ad libitum fed, noninfected); infected (I, ad libitum feed, 250,000 oocysts *S. cruzi* per os), paired (PF, noninfected, fed previous day's intake of a conspecific calf); GH-treated (C_{GH} , 12.5 mg/d pituitary-derived USDA-bGH-B1, im, ad libitum fed); GH-infected (I_{GH} , 12.5 mg/d GH, 250,000 oocysts, ad libitum fed) and pair-fed GH (PF_{GH} , noninfected, 12.5 mg/d GH, paired intake to conspecific I_{GH}). Control, I and PF were injected daily with the GH diluent, 0.05M bicarbonate/carbonate buffer, pH 8.8. There were five animals initially assigned per treatment. Final number of calves per treatment varied between four and five per group at the end of the study because four calves were removed from the study (one/group in C, PF, C_{GH} , I_{GH}) because of the untimely random occurrence of rumen bloat, not uncommon in this age calf fed diets with highly degradable carbohydrate. Because this condition was not observed before Day 35 relative to infection, feed intake metabolite and hormone data through Day 35 was retained for analysis for all calves. Oral dosing with the organism in I and I_{GH} was designated as day 0 of infection. GH injections were initiated on Day 20 post-infection (PI), approximately 3–4 d before the onset of the APR. The rationale for starting GH before the onset of the APR stemmed from previous experiments in our laboratory. Pretreatment of calves with GH for 3 to 5 d reduced the severity of physiological response to endotoxin (16,17). GH injections were continued through 56 dPI. On Day 56 calves were euthanized for total carcass dissection and quantification of tissue composition.

Body Composition Determination. Comparative tissue balance (18) was used to estimate the chemical composition of carcass of calves in the six treatment groups. To accomplish this, an additional five calves were reared under similar conditions and euthanized at an age and weight corresponding to those of calves in the experimental groups on Day 0 of the initiation of GH treatments (corresponding to Day 20 relative to infection). Total carcass dissection of this initial group was performed to calculate the mean weight or mass of a given tissue component (weight, moisture content, percent protein, percent fat, and percent ash) as a percentage of live body weight. Knowing the measured Day 0 starting weight of each experimental calf, we used regression to calculate the Day 0 tissue component for each experimental calf in the six treatments. After removal of hide, head, hooves, and viscera, the carcass was split longitudinally through the vertebral column and one half the carcass ground to a uniform consistency. Three 2-kg aliquots of this were obtained to estimate moisture content by freeze-drying. Representative samples of the freeze-dried material were composited, further ground to a fine powder in the presence of dry ice, the dry ice sublimated, and the product subjected to nitrogen analysis (duplicate 3-g samples) using infrared detection of nitrogen oxide combustion products (Leco Nitrogen Analyzer, Leco Co., St. Joseph, MI) or fat determination using petroleum ether extraction (2-g sample). Ash determination was performed by heating composited tissue to 565°C for 24 h and analytical weighing. The difference between the final component mass and the estimated starting component mass divided by days on treatment (35 for the days of injection of GH or buffer) was calculated to obtain the average daily gain of specific tissue components such as protein and fat. Additional

measurements of average daily weight gain, feed intake, carcass protein gain, plasma urea N content (PUN), and plasma nonesterified free fatty acids (NEFA) were performed. PUN and NEFA were measured as reported previously (17).

Blood Sampling Protocol/Pituitary Tissue Processing. Relative to 20 d PI, daily jugular vein blood samples were collected into EDTA-containing syringes (Sarstedt, Germany) from all calves through Day 28 postinfection. Weekly blood samples were further obtained through Day 56 PI. The pituitary GH responsiveness to provocative secretagogue challenge was tested during the recovery phase (Day 50 post infection) following the APR by jugular vein administration of growth hormone releasing hormone (GHRH, 0.2 mg/kg, bovine peptide 1-29, Sigma, St. Louis, MO) and collection of blood samples for plasma at time 0, 5, 10, 15, 20, 30, 45, and 60 min relative to GHRH administration. For comparison of treatment effects, the area under the concentration time curve was calculated by trapezoidal summation and the baseline area between time 0 and 60 min subtracted from the total area. In order to ascertain the effects of GH, infection and feed intake on pulse-secretion characteristics of GH, indwelling Teflon[®] cannulas (Abbot Labs, Inc, N. Chicago, IL) were aseptically inserted and maintained in the external jugular vein of each calf on day 50 PI. Blood samples (7 ml) were collected at 10-minute intervals into EDTA tubes for a 4-h sampling window beginning at 0800 h. Plasma obtained after centrifugation of blood at $1850 \times g$ for 20 min was stored at -20°C until assayed for GH and IGF-I by radioimmunoassay. Pulse-secretion parameters for GH (baseline concentration, smoothed mean, number of pulses, pulse amplitude) were determined using the PULSAR algorithm as validated and published previously (6). Pituitaries were harvested at slaughter within 5 min of exsanguination. After removal of neurohypophysial tissue, anterior pituitaries were weighed, hemisected, immersed into liquid N, and stored frozen at -80°C . Tissue processing for determination of GH mRNA is described below. For measurement of pituitary GH content, one hemipituitary was thawed and homogenized in 0.1N HCl, ice cold, at a ratio of 1:10 (w/v). The GH-containing extract was serially diluted (1:2) in RIA assay buffer with appropriate adjustments in pH and assayed for GH content by RIA.

Radioimmunoassays for Hormones. Plasma concentrations of IGF-I were measured by double antibody RIA as previously described (6) using UBK-487 antihuman IGF-I as the primary antibody (NIADDK, Bethesda, MD), recombinant human IGF-I (Gro-Pep, Ltd., Australia) as standard and tracer, and glycyl-glycine HCl acidification to pH 3.3 as the plasma pretreatment. Recovery of IGF-I added to plasma before acidification and assay was 92% under the conditions of assay, comparable to that obtained in our laboratory in a limited comparative study of recovery using 1 M acetic acid Sephadex G-50 gel chromatography. Parallelism and recovery were tested using plasma from both normal and infected calves in light of the documented differences in IGF binding proteins present in normal and infected calves (6). Inter- and intra-assay coefficients of variation were 9% and 6%, respectively. Plasma GH was measured as described previously (6) using rabbit antiovine GH serum R-1-1-3 and USDA bGH-B1 as the standard and iodinated for tracer. Inter- and intraassay CVs were 12% and 9%, respectively. Plasma concentrations of TNF- α were measured in a single radioimmunoassay as validated by Kenison et al. (9). Intraassay variability was 10.8% for duplicate samples assayed and recovery averaged 93%.

Hepatic GH Receptor Binding. Hepatic microsomal membranes were prepared by a combination of optimized procedures (19–21) using exposure to 4M MgCl_2 (22) to eliminate endogenous-bound GH before competitive binding studies and traditional Scatchard analysis (23). Hepatic tissue (1–2 g) was homogenized by Polytron (Brinkman Instruments, Westbury, NY) at ice temperature in 0.025M TRIS with 10 mM Ca^{++} , 0.3M

sucrose, and 1,000 KIU/ml aprotinin at pH 7.6 (3:1 volume buffer to weight tissue). Homogenates were centrifuged at $1000 \times g$ for 10 min and supernates retained. Supernates were further centrifuged at $100,000 \times g$ for 2 h at 4°C and the pellet retained. To the pellet was added 300 ml 4M MgCl_2 and this was mixed with a glass rod and triturated through a 21-ga. needle on syringe. After 10 min this mixture was diluted to 5 ml with TRIS- Ca^{++} buffer and centrifuged at $40,000 \times g$. The pellet was washed twice in buffer, resuspended in buffer, and protein content measured by bicinchoninic acid- Cu^{++} binding (BCA, Pierce, Rockford, IL) after triton X-100 and trichloroacetic acid treatment and solubilized with NaOH. GH (USDA-bGH-B1) was iodinated via iodogen (Pierce, Rockford IL) achieving 50,000 cpm equivalent to 3.2 ng bGH. For the assay, 50,000 CPM ^{125}I -bGH was added to 500 mg membrane protein in triplicate with additions of nonlabelled bGH between 0 and 150 ng/tube in a final volume of 400 μl in polypropylene microfuge tubes. The tubes were incubated overnight at 4°C . Separation of bound and free tracer was achieved by addition of 1.0 ml additional buffer and centrifugation at $14,000 \times g$ for 10 min. Tips of tubes were cut off and the radioactivity counted. Nonspecific binding was determined in the presence of 10 mg nonlabelled bGH. Data were expressed as percent specific binding and number of specific receptors/mg protein as determined from the LIGAND analysis (23). For the LIGAND program initialization, values of 1×10^{11} , 3×10^{-13} and 0 were used for K_{11} , R_1 , and N_1 , respectively, as determined empirically from preliminary plotting using simple linear regression.

Messenger RNA Analysis. Tissues were homogenized while maintained on ice using a Polytron. Tissue homogenates were immediately extracted using a guanidinium-thiocyanate-phenol-chloroform procedure (24) to obtain total RNA using RNazol (Tel-Test, Friendwood, TX). Liver was extracted three times and the resulting matrix pooled for subsequent isolation of poly(A)-RNA (PolyATtract, Promega, Madison, WI). Poly (A)-RNA (10 mg/lane) was loaded onto 1% agarose gels and separated electrophoretically for approximately 2 h at 50 V. Messenger RNA was transferred to Nytran[®] (S and S, Keen, NH) by capillary movement overnight and prehybridized for an additional 24 h. Blots were probed for IGF-I mRNA or GH-receptor mRNA using specific cDNA probes kindly donated by Dr. F. Simmons or Dr. W. Warren, respectively. Probes were labelled with ^{32}P to at least 5×10^8 cpm/mg DNA using a random primer kit (Prime-A-Gene Kit, Promega, Madison, WI). Northern blots were hybridized for 72 h using approximately 3×10^6 cpm labelled probe/blot, then washed three times in 2X SSC, 0.1% SDS at room temperature for 15 min each, blotted to remove excess moisture, and then placed on X-ray film for 3–6 d in the case of GH receptor, or 7–10 d for IGF-I message. The intensity of the resolved bands was estimated using scanning laser densitometry. For the purpose of normalization, each blot was stripped of the respective probe and reprobbed using radiolabelled b-actin cDNA. Pituitary GH mRNA was analyzed by Northern blot using 4 mg total mRNA (25,26). The GH cDNA was obtained from P. Rotwein (Washington University, St. Louis, MO) and a ribosomal RNA probe (p28, R. C. Bird, Auburn Univ. AL) used to control for loading differences.

Statistical Analysis. ANOVA or ANOCOVA were performed using a general linear model (SAS, PROC GLM, 27) designed as a 3×2 factorial arrangements of treatments. Infection, GH and pairfeeding were set as main effects within the model and appropriate interactions tested. Because of missing data cells, least squares means and appropriate SEs were calculated and presented in graphs and tables. Body growth and composition data were analyzed using initial body weight as a covariate to adjust for slight differences in initial weight. Plasma concentrations of IGF-I were analyzed using the plasma concentration measured relative to Day 20 postinfection as a covariate. Where main effects were

TABLE 1. EFFECTS OF PARASITIC INFECTION AND PAIRFEEDING ON RECTAL TEMPERATURE, FEED INTAKE AND PLASMA UREA, N AND PLASMA CONCENTRATIONS OF TNF- α AVERAGED ACROSS THE TIME PERIOD ENCOMPASSING ONSET OF ACUTE PHASE RESPONSE THROUGH THE TERMINATION OF THE GROWTH TRIAL

Variable	-GH			+GH			SEM	Effect of Infection Beyond Nutrition ¹	Effect of Nutrition	Effect of GH
	C	I	PF	C	I	PF		P=	P=	P=
Mean Rectal Temp., °C	38.8 ²	39.9	38.5	38.9	40.0	39.0	0.17	0.004	0.84	0.54
Mean Feed Intake, kg/d	4.2	3.3	3.3	4.3	2.8	2.8	0.54	0.04	—	0.11
Plasma Urea N, mg %	10.7	13.6	12.2	9.3	12.9	9.3	0.78	0.009	0.09	0.04
Plasma TNF- α , pg/ml	80.0	105.2	104.8	72.0	108.6	86.1	6.81	0.1	0.001	0.19 (GH \times Nutr. 0.009)

¹ Effects of infection were statistically separated from those of undernutrition, as represented by the pair-fed groupings, by limiting the comparison to that between only the infected calves and their specific pair-fed noninfected replicate mates using appropriate contrast statements in the GLM. P-values for significant interactions in parentheses where appropriate.

² Values are least squares means of data for each variable with standard error derived from the general linear statistical model. Rectal temperatures and feed intake were recorded daily and averaged. Measurements of plasma urea N and TNF- α were obtained daily from onset of APR through Day 28 relative to inoculation with the parasite and thence weekly and averaged.

significant, separation of means was performed using specific orthogonal contrasts. Significant effects were present with $P < 0.05$ and trends suggested with $P < 0.1$.

RESULTS

All calves within groups were similar in weight gain during the period of time preceding initiation of GH or excipient buffer injections. Acute phase response and the presentation of clinical signs were rapid in onset in individual calves between days 23 and 25 PI. Over the next 7–10 d, more severely affected calves displayed decreased packed cell volume, hemolytic anemia and increased plasma concentrations of bilirubin (data not presented). Changes in voluntary feed intake, rectal temperature, and PUN are summarized in Table 1. The overall effect of infection ($P = 0.04$) was apparent in the decline in voluntary intake that began with the onset of the APR. When more specifically compared with ad libitum fed C and C_{GH} calves, total voluntary feed intake was depressed 25% in I ($P < 0.05$ vs C) and 37% in I_{GH} ($P < 0.01$ vs C_{GH}; $P < 0.1$, I vs I_{GH}). Infected calves displayed increased rectal temperatures initially measured between Day 23 and 25 PI. Increased rectal temperature was apparent in I and I_{GH} for at least 21 consecutive days in the observation period ($P = 0.004$); there was no tendency for GH treatment to affect the magnitude or duration of the fever response. Peak temperatures of infected calves were recorded on Days 27 and 28 PI and averaged at this time $40.7 \pm 0.1^\circ\text{C}$. For the 2-wk period between Day 24 and Day 38 postinfection, the average rectal temperature of infected calves was 0.94°C higher than the control and pair-fed calf means. There was no difference in rectal temperatures between GH-treated and non-treated calves. Plasma concentrations of urea N also increased with the onset of APR in infected calves and to a lesser extent in pairfed calves. Overall treatment with GH decreased PUN 1 to 2 mg/dl across calf groups compared with non-GH-treated calves ($P = 0.04$) but GH failed to blunt

TABLE 2. EFFECTS OF INFECTION, DAILY GH TREATMENT, AND PAIRFEEDING ON SPECIFIC WHOLE BODY, CARCASS, VISCERAL ORGAN, AND MUSCLE GROWTH ATTRIBUTES IN CALVES

Variable	-GH			+GH			SEM	Effect of Infection Beyond Nutrition ¹	Effect of Nutrition	Effect of GH
	C	I	PF	C	I	PF		P=	P=	P=
Av. Daily Gain, kg/d	1.39 ²	0.95	1.06	1.51	0.79	0.73	0.08	0.80	0.01	0.12
Av. Daily Carcass Gain, kg/d	0.64	0.36	0.54	0.64	0.35	0.27	0.05	0.02	0.01	0.15 (GH×Nutr. 0.02)
Av. Daily Carc. Prot. Gain, g/d	128.9	51.8	104.7	121.8	47.8	66.7	12.2	0.005	0.005	0.12
Av. Daily Carc. Fat Gain, g/d	93.1	11.2	37.3	67.9	-22.7	27.6	12.3	0.01	0.01	0.03
Carcass Ash, %	10.29	12.03	10.92	11.20	12.00	10.07	1.2	0.1	0.12	0.69
Av. Daily Intestinal Prot. Gain, g/d	3.19	3.04	2.22	4.13	0.21	1.22	0.1	0.91	0.03	0.24 (GH × INF 0.05)
Av. Daily Intestinal Fat Gain, g/d	39.2	10.5	11.1	40.2	5.8	10.9	6.8	0.66	0.01	0.87
Rectus Femoris Moisture, %	77.51	78.60	78.3	77.7	79.0	77.8	0.3	0.002	0.02	0.62
Av. Daily R. Femoris Prot. Gain, g/d	1.97	1.17	1.68	1.64	1.51	1.11	0.3	0.33	0.15	0.46
Psoas major Moisture, %	76.7	78.5	77.7	76.7	78.3	77.1	0.3	0.001	0.05	0.40
Av. Daily P. major Prot. Gain, g/d	1.03	-0.08	0.87	1.61	0.21	0.51	0.2	0.01	0.05	0.1

¹ Effects of infection were statistically separated from those of undernutrition, as represented by the pair-fed groupings, by limiting the comparison to that between only the infected calves and their specific pair-fed noninfected replicate mates using appropriate contrast statements in the GLM. P-values for significant interactions in parentheses where appropriate.

² Values are least squares means of data for each variable with S.E. derived from the general linear statistical model.

the rise in PUN during APR in infected calves ($P = 0.009$). Mean plasma concentrations of TNF- α were increased to concentrations numerically greater than control and preinfection levels after the onset of the acute phase response to the pathogen. The overall mean response was possibly associated with a change in nutrition ($P = 0.001$) because the concentrations measured in the infected calves did not differ from that in the noninfected pairfed animals. The concentrations in these calves were greater than those measured in C or C_{GH}. However, the initial increase in plasma TNF- α occurred earlier in time in infected calves compared to their pairfed conspecifics and toward the end of the grow-out period, remained elevated in the infected calves. TNF- α concentrations in the pairfed calves tended to decrease with the resumption of normal feed intakes. There was a significant GH \times nutrition interaction suggesting that some intricate relationship in the modulation of plasma TNF- α occurs as metabolism is modified by GH in animals consuming different levels of feed.

Growth characteristics of calves infected with *S. cruzi*, their pairfed conspecifics and the effects of treatment with GH is summarized in Table 2. Whole-body average daily weight gain was decreased in infected and pairfed calves compared to controls. Statistically, all of the observed effects were associated with the reduced nutrient intake of infected calves ($P = 0.01$). GH had no effect on daily body weight gain. Rates of weight gain of the carcass was more specifically affected than whole body weight by both

infection and nutrition. Average daily carcass weight gain was reduced an average of 38% between treatments as a function of reduced nutrition alone ($P = 0.01$). Daily carcass gain was decreased an average of 46% in infected calves as compared to controls ($P < 0.001$). In addition, carcass gain in infected calves was significantly lower than that estimated in their pairfed conspecifics ($P = 0.02$). Components of the tissues were significantly affected by both nutrition and infection. Daily carcass protein gain was decreased on average 61% in both GH-treated and nontreated infected calves. GH had no potential to alter the magnitude of the decreased protein accretion in the total tissue. Average daily carcass fat accretion was affected by nutrition, infection, and the administration of GH. The reduction in fat accretion rate was lower than that of pair-fed conspecifics by 70% in non-GH-treated calves and 90% in calves treated with GH. Carcass fat gain of I_{GH} was significantly lower than that of I , suggesting an additive effect of infection to mobilize fat (or decrease synthesis) in the presence of GH. Significant GH \times infection interactions were present for average daily carcass gain ($P = 0.02$) and associated with this an interaction on the utilization of feed for carcass gain (carcass gain efficiency, $P = 0.045$, data not shown). Intestinal tissue balance was significantly influenced by nutrient intake level but beyond that, refractory to the effects of infection. Analysis revealed a significant GH \times infection interaction ($P = 0.05$) for intestinal protein gain wherein a large decrease in intestinal protein gain in infected animals treated with GH was observed. A significant effect of reduced nutrient intake on intestinal fat gain was evident.

Isolated and dissected in toto, rectus femoris and psoas major muscles were chosen as representative muscles of locomotion and posture, respectively. The data illustrate that GH had no apparent effect on daily protein gain in rectus femoris of normal or infected calves but also the muscle was relatively refractory to the catabolizing effects of infection. Psoas major, on the other hand, responded to GH with a trend toward increased daily protein gain and a capacity to maintain a higher level of protein accretion during infection in the presence of GH but was highly sensitive to the presence of infection and displayed a net average negative protein accretion for the test period. Tissue edema within muscle in response to invasion by the terminal stage of the parasite and development of sarcocysts is reflected in the increase in moisture content of rectus femoris ($P = 0.002$) and psoas major ($P = 0.01$) beyond that which apparently developed in association with altered nutritional intake ($P = 0.02$, rectus femoris; $P = 0.05$, psoas major).

The change in plasma IGF-I concentrations in calves during treatment with GH or saline and the effects of parasitic infection and pairfeeding are presented in Figure 1a, b. The change in plasma concentration (increment or decrement) was related to the plasma concentration measured on the day immediately initiation of GH or bicarbonate buffer injections. Before the onset of APR and initiation of GH treatments, plasma IGF-I averaged 117 ± 5 ng/ml across calf groups and did not differ between groups. Within 24 hours of the start of GH injections, plasma IGF-I increased ($P < 0.02$) and remained increased in all GH-treated calves until the onset of fever and the APR. Plasma IGF-I in infected calves decreased steadily to a maximum nadir between 26 and 30 d PI. The decline in plasma IGF-I in I and I_{GH} preceded the decline that developed in PF and PF_{GH} by 1–2 d; the decrease in plasma IGF-I in the pairfed calves was always less than that measured in the infected conspecific calf. The ability of GH to maintain increased plasma concentrations of IGF-I was compromised severely in all infected calves and blunted in response to decreased intake of pairfed calves.

The relationship between the decrement in plasma IGF-I (the difference in concentration measured on the day preceding relative onset of APR to maximum nadir) and a measure of the intensity of the response to infection (maximum change in rectal temperature) is presented in Figure 2. Although all infected animals displayed clinical signs of

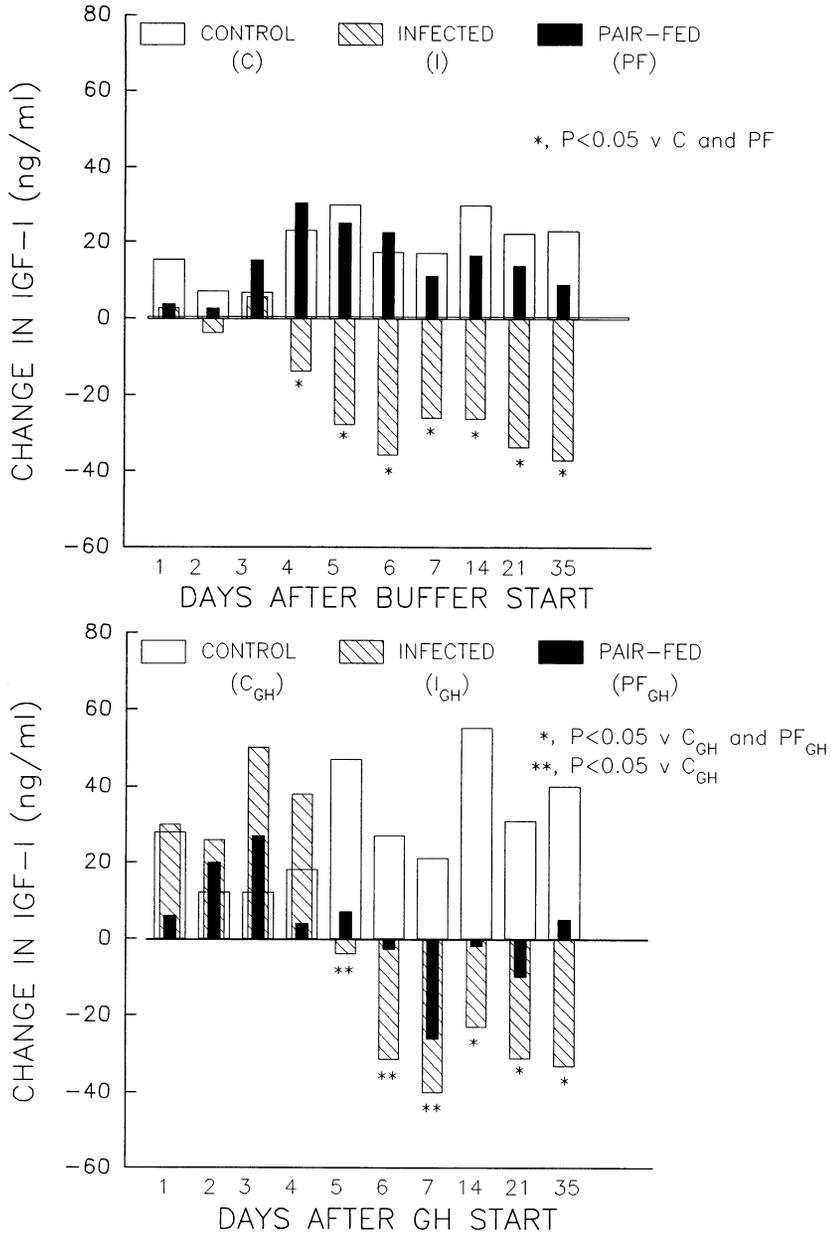


Figure 1. Effects of infection, paired nutrient intake and GH on changes in plasma concentrations of IGF-I during the time course of infection in calves. Data are least squares means of four to five calves/group. Data are least squares means (ng/ml) for the change in plasma IGF-I on a stated day compared with levels measured on Day 20 relative to the start of infection and 3 to 4 days before the onset of acute phase response (Day 3–4 of buffer or GH injection). Pooled S.E. = 12 ng/ml. Top panel: responses in control, infected and pair-fed calves treated with buffer excipient. Bottom Panel: responses of control, infected and pair-fed calves treated daily for 35 d with 12.5 mg/h/d USDA-bGH-B1.

APR, animal-to-animal variation in response was large. Regression relationship between change in temperature (independent variable) relative to the change in IGF-I (dependent variable) demonstrated that there was a negative correlation, highly significant ($P < 0.01$) and linear. Calves responding with the greatest increase in temperature correspondingly

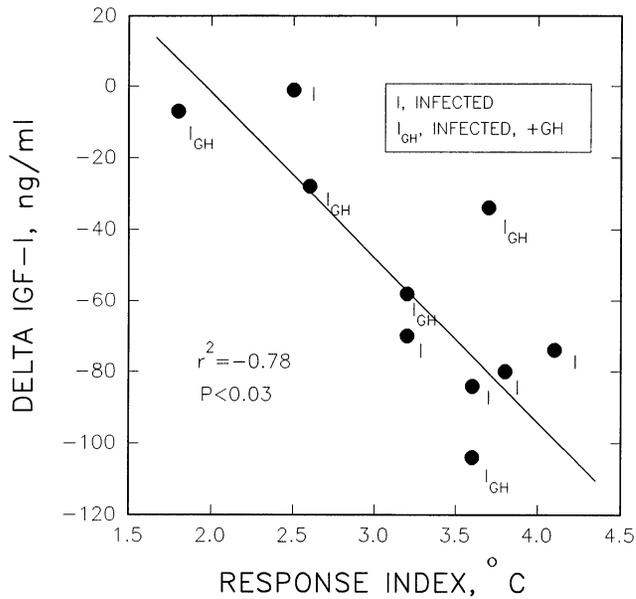


Figure 2. Correlation of fever response index with the decline in plasma concentration of IGF-I in infected calves and infected calves treated with GH.

had the largest decrease in plasma IGF-I. Partial correlations (not plotted) in this relationship were of a similar negative slope whether calves had been treated with GH or not.

Table 3 summarizes data concerning the GH response to GHRH challenge and pulsatile secretory characteristics, capacity of hepatic microsomal membranes to bind ^{125}I -bGH, levels of hepatic mRNA for the GH receptor and IGF-I and the final plasma concentration of IGF-I in calves during the recovery period of the infection. Plasma concentrations of GH were significantly increased by GH injection. Overall treatment of calves with GH resulted in an attenuation of the GH response to GHRH by 39% ($P < 0.03$). There was a trend for the GH response to GHRH to increase with feed reduction in calves not treated with GH but largely no effect of infection or feed intake was significant. In patterns of secretion, the episodic character of GH release was dampened, principally through an attenuation in the frequency of secretory pulses observed in the sampling window ($P < 0.05$). At this point in the time course of infection events, neither infection per se nor feed intake significantly affected GH secretory characteristics.

The numbers of liver microsomal GH binding sites (Table 3) were not different among infection or nutritional or GH treatments. In a generalized theme, values for the concentration of GHR were numerically lower in preparations from calves consuming reduced nutrition (infected or not), but the differences were not significant relative to control animals. Interestingly, mRNA levels for GHR were significantly lower in infected calves than those measured in controls of paired animals. Similarly, hepatic mRNA for IGF-I was significantly lower in infected calves than other animals. Terminal plasma concentrations reflected the low levels of IGF-I mRNA in infected calves. At this time in the study, infected calves were consuming 80% or greater amounts of feed relative to ad libitum fed calves. This slight change in feed intake corresponded to a trend for lower plasma IGF-I in paired calves but a maintained significant decreased concentration in infected animals. Regression relationships suggested a positive correlation between hepatic IGF-I mRNA levels and final plasma IGF-I concentrations in non-GH-treated calves

TABLE 3. EFFECTS OF GH TREATMENT, PARASITIC INFECTION, AND PAIR-FEEDING ON PLASMA GH SECRETORY CHARACTERISTICS AND LIVER MICROSOMAL GROWTH HORMONE RECEPTOR (GHR) BINDING CHARACTERISTICS, mRNA, PLASMA CONCENTRATIONS OF IGF-I, AND HEPATIC IGF-I mRNA IN CALVES AT STUDY TERMINATION

Variable	-GH			+GH			SEM	Effect of Infection Beyond Nutrition ¹	Effect of Nutrition	Effect of GH
	C	I	PF	C	I	PF		P=	P=	P=
Av. Plasma GH (ng/ml)	6.1 ²	6.5	7.2	10.0	8.4	9.7	.8	0.54	0.19	0.15
No. Peaks	2	1	1	0	0	0	1	0.67	0.32	.05
Peak Amplitude (ng/ml)	5.4	12.1	5.8	6.0	3.4	6.1	2.4	0.41	0.31	0.14
GH Response to GHRH (area units)	1581	1101	1664	1557	991	1466	220	0.05	0.52	0.14
Pituitary GH content (ng/mg)	12.5	12.5	14.0	8.6	8.8	13.1	2.6	0.36	0.49	0.24
Pituitary GH mRNA GH; p28 (rRNA)	0.91	1.00	1.03	1.12	0.83	0.80	0.20	0.79	0.45	0.23
GHR, -13 ($\times 10^3$ M)	5.2	2.6	2.4	3.4	2.5	2.5	1.3	0.93	0.18	0.67
GHR mRNA GHR:Actin	2.2	1.7	2.3	1.7	1.3	2.9	0.6	0.05	0.80	0.77
PLASMA IGF-I, (ng/ml)	142	94	138	161	98	110	7	0.002	0.015	0.1
IGF-I mRNA, (IGF-I:Actin)	1.5	0.8	1.4	1.5	0.6	1.6	0.4	0.041	0.35	0.94

¹ Effects of infection were statistically separated from those of undernutrition, as represented by the pair-fed groupings, by limiting the comparison to that between only the infected calves and their specific pair-fed noninfected replicate mates using appropriate contrast statements in the GLM. P-values for significant interactions in parentheses where appropriate.

² Values are least squares means of data for each variable with standard error derived from the general linear statistical model. Tissues and plasma samples were collected at time of euthanasia. C, Control; I, Infected; PF, paired to a specific I.

(data not presented). No correlation was present for the same relationship in GH-treated calves. Occasionally infected calves had a high hepatic mRNA level for IGF-I in spite of low plasma concentrations. Similarly, there was no statistically significant correlation between mRNA levels for GHR and mRNA levels for IGF-I.

DISCUSSION

In the present study, the level of parasitic challenge was chosen to represent a moderate infection that might be responsive to modulation by exogenous treatments. A significant degree of animal variability in the response to the disease challenge was apparent. Where comparisons of reported means may have suggested responses of only trend significance, the regression data analysis strongly demonstrated the presence of a gradient of response in that animals more severely affected by infection were more severely compromised in their ability to maintain lean tissue mass and IGF-I profiles. Whereas Beisel (28) suggested in general terms that the overall magnitude of systemic response to a stress was proportional to the severity of the insult, the data herein are, to the best of our knowledge, the first to demonstrate a quantifiable correlation between a measurable index of response

(fever) to an active infection and the magnitude of change in plasma IGF-I levels, a marker of importance in the overall patterns of nutrient use and nitrogen balance.

The present study demonstrates that daily treatment of young calves with bovine GH is without merit in preventing the reduced lean tissue accretion and plasma concentrations of IGF-I associated with the cachexia of parasitic infection. Biological activity of the GH used for treatment was confirmed by the increase in plasma IGF-I in all calves before the onset of the APR, overall lower PUN concentrations in GH-treated calves, and decreased tissue accretion rates of fat.

Previous data from our laboratory demonstrated a beneficial effect of GH pretreatment to ameliorate the severity of metabolic perturbations modeled with endotoxin challenge (16,17). In those studies GH treatment was associated with a decrease in the magnitude of TNF- α and glucocorticoid concentrations in plasma as well as blunting of metabolite responses mostly associated with metabolic stress. Data in the present study conflicts with those earlier findings. In the present study, the effect of GH was largely confined to an overall decrease in plasma concentrations in pair-fed calves, as apparent in the significant GH-by-nutrition interaction. In this study, a major portion of the modest increase in circulating plasma concentrations of TNF- α occurred in association with a reduction in feed intake in non-GH-treated calves. Thus the patterns of TNF- α altered in the presence of this parasitic infection were atypical of those evidenced with the administration of endotoxin or bacterial sepsis. The lack of agreement may be related to the age of animals used, duration of GH treatment, breed and body composition at the time of challenge. More likely, however, are the realities that (a) the endotoxin challenges previously used in the study where GH pretreatment had a beneficial effect were quite mild in comparison to the severity and duration of metabolic and immune reaction imposed with parasitic challenge used here, (b) the localized tissue TNF- α response in this infection might be typical of a paracrine response, thus limiting the utility of the measurement in plasma cytokines during this time period, and (c) some of the variability complicating the TNF- α data could have been related to animal-to-animal variation in the relative proliferation of the organism in the host and therefore the magnitude of the cytokine response. The fact that plasma concentrations of TNF- α were affected by the plane of nutrition is consistent with the previous observations by Kahl et al. (29) demonstrating that moderate changes in protein intake by cattle affects the magnitude of the TNF- α response to low-level endotoxin challenge.

Recent data from Liao et al. (30) demonstrated that GH potentiated the severity of the *in vivo* biological response to endotoxin by rats. GH is known to increase oxidative radical production in response to endotoxin and cytokine challenge (31). In addition, in the most severe responses to endotoxin, nitrogen-free radicals generated through the inducible nitric oxide synthase pathway can combine with superoxide to produce peroxynitrite and cause extensive intracellular lipid and protein oxidative damage (32) contributing to multiorgan failure and metabolic and neurological complications. Data from Edwards et al. (33) suggested that GH treatment of hypophysectomized rats resulted in increased TNF- α release from macrophages harvested from these animals when the cells were challenged *in vitro* with endotoxin. However, they also demonstrated increased survival of GH-replenished rats challenged with bacterial infection. The differences in findings between studies, suggest that the effects of GH on the response to a disease stress may need to be defined relative to the specific nature of the disease challenge and possibly to the magnitude of the challenge and host response. A similar lack of effect of GH treatment to a different parasitic infection (*Eimeria bovis*) model has also been described (34).

Boosting the somatotrophic axis by means of either GH or IGF-I administration has been suggested as beneficial effects in conditions of metabolic wasting associated with under-

nutrition and some states of trauma. Clemmons and Underwood observed significant increases in N retention in underfed patients treated with either GH or IGF-I (13,14) and Ogawa et al. (35) suggested that pretreatment of lambs with bovine GH was effective in partially offsetting the nitrogen losses associated with fasting. Similarly, Douglass et al. (36) suggested that direct administration of IGF-I was effective in ameliorating some of the catabolic effects of direct infusion of recombinant TNF- α , a significant mediator of metabolic wasting in disease stress (37). However, it was observed as early as 1988 that septic patients were refractory to GH therapy with regard to both nitrogen retention and plasma IGF-I effects of GH (38,39). More recently, we have modeled the decrease in plasma IGF-I during endotoxemia in calves showing that the decrease in IGF-I after endotoxin was not accounted for by comparable levels of decreased feed intake in pair-fed conspecifics (40). Furthermore, data in that study suggested that IGF-I may be differentially partitioned to tissues depending on the changes in IGF-binding protein profiles supporting the data reported similarly in the rat by Fan et al. (41).

Our initial investigations into the cause of growth stunting in similarly infected calves demonstrated that the pituitary itself was a target for the effects of acute phase response mediators during sarcocystis infection (5,6). In these early studies, secretory characteristics of GH were significantly attenuated by the presence of infection as were GH responses to dynamic challenge with thyrotropin releasing hormone. It was suggested at that time that the pituitary GH dysfunction may have played a significant role in the prolonged decrease in circulating IGF-I. The data in the present study clearly indicate that IGF-I concentrations remain depressed in infected calves through 56 d postinfection. However, mean circulating plasma concentrations and patterns of GH at this time do not suggest that a GH deficit accounts for decreased IGF-I. Still, GH administration was effective in blunting the GH response to GHRH, an event attributed to the direct negative feedback effects of the exogenous GH treatment and somewhat indicative of functionality of the GRH in hypothalamic and pituitary tissues.

In conveying a biological message to a tissue, hormone action may be modulated by the activity of the receptor rather than just the concentration or pattern of hormone presentation to the tissue. A lasting GH receptor dysfunction may contribute to the observed IGF-I deficit and growth stunting mechanism. Messenger RNA for GH receptor as well as mRNA for hepatic IGF-I were significantly decreased specifically by infection. Interestingly, characteristics of hepatic binding of ^{125}I -bGH to homologous hepatic microsomal membrane preparations were not different between animal groups. Binding data reduction program used in this experiment suggested a single class of high affinity receptor, in contrast to earlier reports of multiple classes of GH receptors that were nutritionally dependent in calves (20).

We observed considerable variability between animals in receptor content of microsomes that made overall interpretation of a discrete perturbation difficult to resolve. The recent report by Ogawa et al. (35) in lambs similarly failed to show a significant effect of fasting on overall binding of radiolabelled bGH to ovine hepatic microsomal membranes. The lack of difference in GH receptor characteristics across treatments in this study suggests that the problem in IGF-I regulation may be distal to the binding of the GH to its receptor. Conversely, by using the MgCl_2 stripping technique, we ultimately measured total microsomal GH receptors. Perhaps a measure of the endogenous saturated/unsaturated receptor ratio would have been more definitive and consistent with the effects of infection on GHR mRNA.

The effect of the infection on GH-regulated processes could be distal to the GH binding event or relate to differential effects across binding in several tissues. The effects observed in liver may not represent all of the possible mechanisms through which this infection

impacts GH regulation of IGF-I. Research is currently underway to study aspects of GH-receptor signal transduction interruption as a possible site for somatotrophic axis dysfunction in this disease.

The present study confirms and further extends earlier reports from our laboratory on this IGF-I deficit effect (6). The present data demonstrate that the decrease in plasma IGF-I in infected animals during APR preceded that of pair-fed conspecifics by as much as three days and was always more severe than could be accounted for by the degree of reduced nutritional intake. This point is particularly significant in that the pair-fed calves in this study can not truly be considered in a state of nutritional stress, as might be associated with poor nitrogen balance and weight gain, when rates of live weight gain greater than 1 kg/d were in evidence. In addition, there was suggestion that the presence of the GH treatment may have delayed the decline in plasma IGF-I in infected calves as evidenced by the 1-to-2-d difference in the time at which a valid decrease in IGF-I was observed. However the chronic nature of the events causing the downregulation of IGF-I still resulted in a long-term decline in plasma IGF-I. Thus, the failure of infected calves to maintain IGF-I levels from the onset of APR, even when treated with GH, is not simply a matter of uncoupling the somatotrophic axis regulation from GH as a function of nutritional stress, as has been suggested to occur in several nutritional reports (42,43). Short-term down regulation of growth and aspects of somatotrophic axis-directed metabolism may be a necessary part of the survival response early in the onset of APR (44). However, usually this down regulation is transient and may reflect an adaptation period to conserve nutrients for metabolic purposes of higher survival priority than growth. Sarcocystosis seems to be a disease in which the somatotrophic axis down regulation becomes a pathology in its own, setting a metabolic path to promote accelerated tissue wasting.

Difficulty was encountered in making conclusions regarding many aspects of effects of infection on responses of tissues. Basically three pools of tissue subcomponents, namely, protein, moisture, and fat, were differentially affected by infection. Lean tissues, predominantly psoas major and carcass muscle protein, of infected animals had significantly higher moisture content than that measured in noninfected animals. The higher moisture content could be related to the edema associated with the inflammatory response of the muscle as the sarcocystis parasite invades. In addition, the change in muscle water balance could be related to cytokine-induced sodium and water sequestration as suggested by Kern and Norton (3). Also, most carcass tissues had a lower fat content in association with infection. Intestinal tissue was differentially affected by infection according to whether GH treatment was applied. When calves consumed less feed, rates of intestinal protein and fat accretion decreased compared with controls. However, infected calves treated with GH had a consistent significant decrease in intestinal protein gain without any effect on fat accretion in that tissue bed. Perhaps this decrease in gut protein mass resulted in poor nutrient absorptive character across the gut and further compromised nutrient availability in these calves. The capacity for perturbed gut nutrient uptake in sarcocystis-infected calves was suggested previously by our lab in association with nitrogen balance and digestibility trials (5,6) and altered gut somatostatin levels and molecular forms in association with infection (45). It was interesting to note that infection had a minimal effect on the protein accretion rate of rectus femoris muscle, a muscle in the bovine with locomotor function and a high degree of fast-twitch fiber character. In contrast, psoas major, a postural muscle with greater percentage of b-slow twitch fibers, was actually catabolized to present an overall net negative protein accretion rate. Although the treatment differences were not significant, the data for the psoas major suggested a trend for this muscle to be responsive to positive accretion effects of bGH. Basically, live weight

and average daily weight gains presented a poor reflection of the severity of the cachexia present because the effect of infection and confounding influences of level of feed intake varied so markedly between different tissue beds in terms of the changes in protein, lipid, and water mass.

The effect of bGH to increase weight gain even in noninfected calves at this age and weight was marginal. Pell et al. (46) and Collier et al. (15) suggested that the ability for cattle to respond to treatment with exogenous GH with increases in weight gain and protein accretion was highly variable and significantly below that purported to occur in monogastric species. These summaries suggest that the most obvious increases in weight gain in calves treated with somatotropin were observed in studies where the comparisons were made in groups of calves fed below ad libitum intakes. In one report of a positive effect of GH treatment on whole body weight gain, where calves gained weight at an average rate 80% of that reported here (47), the feeding regimen was less than ad libitum and no significant effect of GH on semitendinosus muscle protein accretion was observed. In respect to some studies, the rates of body weight gain in the present study approached twice those reported in other literature. In this regard, with the levels of intake and nutritional composition of the feed, calves in our study may have been growing at near maximum rates of gain with little room left in the growth curve for GH to boost performance. Added to this is the possibility that the amino acid requirements may have been out of balance for the physiological conditions encountered (48). Additionally, the average 35-d duration of treatment may have been insufficient to permit much more than the 7% increase in weight gain noted. It seems that metabolic effects of GH in the young calf may be present and regulated differently as a function of age. Whereas effects of GH in this age calf on nitrogen and protein metabolism were few, significant effects on lipid accretion were clearly evident. Further research is needed to establish the species relationships that dictate the age-related responses to GH treatment (immunological and metabolic) as well as determine if animals at a GH-responsive age are more protected against tissue losses associated with disease stress.

Collectively, these data demonstrate that GH treatment was ineffective as a therapeutic measure to aid in stabilizing or minimizing the general tissue wasting present in a cachexia induced by a systemic parasitic infection as modeled in the young calf. These data are consistent with and extend the observations of Bentham et al. (49), suggesting that patients with hypercatabolism diseases acquire resistance to GH. The data suggest that across several body tissue pools, some are more susceptible to catabolism than others, and that some specific muscle types that may be affected with GH treatment are not spared during cachexia. More research on age and species effects on the ability of GH to aid in metabolic perturbations of active infections need to be performed to better define the potential benefits and limitations to this approach to disease intervention.

REFERENCES

1. Giacosa A, Frascio F, Sukkar SG, Ronsella S. Food intake and body composition in cancer cachexia. *Nutrition* 12:s20-s23, 1996.
2. Truyens C, Torrico F, Angelo-Barrios A, Lucas R, Heremans H, De Baetselier P, Carlier Y. The cachexia associated with *Trypanosoma cruzi* acute infection in mice is attenuated by anti-TNF- α , but not by anti-IL-6 or anti-IFN- γ . *Parasite Immunobiol* 17:561-568, 1995.
3. Kern KA, Norton JA. Cancer cachexia. *J Parent Ent Nut* 12:286-298, 1988.
4. Fayer R, Lynch GP. Pathophysiological changes in urine and blood from calves experimentally infected with *Sarcocystis cruzi*. *Parasitology* 79:325-336, 1979.
5. Elsasser TH, Hammond AC, Rumsey TS, Fayer R. Perturbed metabolism and hormonal profiles in calves infected with *Sarcocystis cruzi*. *Domest Anim Endocrinol* 3:277-287, 1986.
6. Elsasser TH, Rumsey TS, Hammond AC, Fayer R. Influence of parasitism on plasma concentrations of

- growth hormone, somatomedin-C and somatomedin-binding proteins in calves. *J Endocrinol* 116:191–200, 1988.
7. Heath HL, Blagburn B, Elsasser TH, Pugh DG, Sanders BS, Sartin EA, Steele B, Sartin JL. Hormonal modulation of the physiological responses of calves infected with *Eimeria bovis*. *Amer J Vet Res* 58:891–896, 1997.
 8. Fayer R. Influence of parasitism on growth of cattle possibly mediated through tumor necrosis factor. In: *Biomechanisms Regulating Growth and Development*. G. A. Steffens and T. S. Rumsey, eds. Kluwer Academic Publisher, Boston, p. 437, 1988.
 9. Kenison DC, Elsasser TH, Fayer R. Radioimmunoassay for tumor necrosis factor- α . Concentrations and circulating molecular forms. *J Immunoassay* 11:177–198, 1990.
 10. Kluger MJ. Fever: role of pyrogens and cryogens. *Physiol Rev* 71:93–108, 1991.
 11. Powanda MC. Host metabolic alterations during inflammatory stress as related to nutritional status. *Am J Vet Res* 41:1905–1911, 1980.
 12. Kelley KW. Effects of growth hormone on the immune system. *Proc NY Acad Sci* 257:95–115, 1989.
 13. Clemmons DR, Underwood LE. Nutritional regulation of IGF-I and IGF-binding proteins. *Ann Rev Nutr* 11:393–412, 1991.
 14. Clemmons DR, Underwood LE. Role of insulin-like growth factors and growth hormone in reversing catabolic states. *Horm Res* 38 (suppl 2) 37–40, 1992.
 15. Collier RJ, Vicini JL, Knight CD, McLaughlin CL, Baile CA. Impact of somatotropins on nutrient requirements in domestic animals. *J Nutrition* 122:855–860, 1992.
 16. Elsasser TH, Rumsey TS, Fayer R, Hartnell GF. Recombinant bovine somatotropin blunts plasma tumor necrosis factor, cortisol and thromboxane responses in vivo. *Endocrinology* 134:1082–1088, 1994.
 17. Elsasser TH, Richards M, Rumsey TS, Collier RJ. Growth hormone administration selectively affects physiological responses to repeated endotoxin administration. *Domest Anim Endocrinol* 13:313–324, 1996.
 18. Rumsey TS, Tyrrell HF, Dinius DA, Moe PW, Cross HR. Effect of diethylstilbestrol on tissue gain and carcass merit of feedlot beef steers. *J Anim Sci* 53:589–600, 1981.
 19. Hung CH, Moore WV. Binding of growth hormone to bovine liver membranes. *Mol Cell Endocrinol* 35:151–157, 1984.
 20. Breier BH, Gluckman PD, Bass JJ. The somatotrophic axis in young steers: influence of nutritional status and 17 β -estradiol on hepatic high- and low-affinity somatotrophic binding sites. *J Endocrinol* 116:169–177, 1988.
 21. Haro LS, Collier RJ, Talamantes FJ. Homologous somatotropin radioreceptor assay utilizing recombinant bovine growth hormone. *Mol Cell Endocrinol* 38:109–116, 1984.
 22. Elsasser TH, Caperna TJ, Fayer R. Tumor necrosis factor- α affects pituitary hormone secretion by a direct pituitary interaction. *Proc Soc Exp Biol Med* 198:547–554, 1991.
 23. Munson PJ. LIGAND: A program system for fitting multiple ligand multiple binding site data. BCTIC, Vanderbilt University Medical Center, Nashville, TN, 1981.
 24. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–164, 1987.
 25. Soyoola EO, Burgess MF, Bird RC, Kempainen RJ, Sartin JL. Neurotransmitter receptor agonists regulate growth hormone gene expression in cultured ovine pituitary cells. *Proc Soc Exp Biol Med* 207:26–33, 1994.
 26. Coleman ES, Sartin JL. Endotoxin stimulates in vitro pituitary growth hormone release in eicosanoid-dependent manner. *Amer J Vet Res* 57:1662–1667, 1996.
 27. SAS. SAS/STAT. Guide for Personal Computers. Version 6 Edition SAS Institute, Cary NC, 1985.
 28. Biesel W. The effects of infection on growth, in: *Biomechanisms Regulating Growth and Development*, Volume 12, (G. L. Steffens and T. S. Rumsey, eds.), Kluwer, Boston, p. 395–408, 1988.
 29. Kahl S, Elsasser TH, Blum J. Nutritional regulation of plasma tumor necrosis factor- α and plasma and urinary nitrite/nitrate responses to endotoxin in cattle. *Proc Soc Exp Biol Med* 215:370–376, 1997.
 30. Liao W, Rudling M, Angelin B. Growth hormone potentiates the in vivo biological activities of endotoxin in the rat. *Eur J Clin Invest* 26:254–258, 1996.
 31. Fu YK, Arkins S, Fuh G. Growth hormone augments superoxide anion secretion of human neutrophils by binding to the prolactin receptor. *J Clin Invest* 89:451–457, 1992.
 32. Beckman JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. *Am J Physiol* 271:1424–1437, 1996.
 33. Edwards CK, Lorence RM, Dunham DM, Arkins A, Younger LM, Gteager A, Walter R, Dansour R, Kelley KW. Hypophysectomy inhibits the synthesis of tumor necrosis factor- α by rat macrophages: partial restoration by exogenous GH or interferon. *Endocrinology* 128:989–996, 1991.
 34. Heath H, Blagburn BL, Elsasser TH, Pugh D, Steele B, Sartin JL. Estradiol-progesterone pretreatment antagonizes the development of symptoms associated with *Eimeria bovis* infection. *J Anim Sci* 72 (Suppl. 1):162, 1994.
 35. Ogawa E, Breier BH, Bauer MK, Gallaher BW, Grant PA, Walton PE, Owens A, Gluckman PD.

- Pretreatment with bovine growth hormone is as effective as treatment during metabolic stress to reduce catabolism in fasted lambs. *Endocrinology* 137:1242–1248, 1996.
36. Douglas RG, Gluckman PD, Breier BH, McCall JL, Parry B, Shaw JHF. Effects of recombinant IGF-I on protein and glucose metabolism in rTNF-infused lambs. *Am J Physiol* 261:606–612, 1991.
 37. Butler B, Cerami A. Tumor necrosis factor: A macrophage hormone governing cellular metabolism and inflammatory response. *Endocrine Rev* 9:57–66, 1988.
 38. Dahn MS, Lange MP, Jacobs LA. Insulin-like growth factor-I production is inhibited in human sepsis. *Arch Surg* 123:1409–1414, 1988.
 39. Ross R, Meill J, Freeman E, Jones J, Matthews D, Preece M, Buchanan C. Critically ill patients have high basal growth hormone levels with attenuated oscillatory activity associated with low levels of Insulin-like growth factor-I. *J Clin Endocrinol* 35:47–54, 1991.
 40. Elsasser TH, Caperna TJ, Rumsey TS. Endotoxin administration decreases plasma insulin-like growth factor (IGF)-I and IGF binding protein-2 in cattle independent of changes in nutritional intake. *J Endocrinol* 134:175–185, 1995.
 41. Fan J, Molina PE, Gelato MC, Lang CH. Differential tissue regulation of insulin-like growth factor-I content and binding proteins after endotoxin. *Endocrinology* 134:1685–1692, 1994.
 42. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocrine Rev* 15:80–101, 1994.
 43. Elsasser TH, Rumsey TS, Hammond AC. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. *J Anim Sci* 67:128–141, 1989.
 44. Elsasser TH, Kahl S, Steele NC, Rumsey TS. Nutritional modulation of somatotrophic axis-cytokine relationships in cattle: A brief review. *Comp Biochem Physiol* 116:209–222, 1997.
 45. Elsasser TH, Fayer R, Rumsey TS, Hammond AC. Plasma and tissue concentrations and molecular forms of somatostatin in calves infected with *Sarcocystis cruzi*. *Domest Anim Endocrinol* 7:537–550, 1990.
 46. Pell JM, Gill M, Beever DE. Variability of responsiveness to GH in ruminants: nutrient interactions. In: Sejrsen K, Vestergaard M, Neimann-Sorensen A, ed. *Use of Somatotropin in Livestock Production*, 286–287, Elsevier Applied Science, London, 1989.
 47. Kirchgessner VM, Roth FX, Schams D, Karg H. Influence of exogenous growth hormone (GH) on performance and plasma GH concentrations of female calves. *J Anim Physiol Anim Nutr* 58:50–59, 1987.
 48. Boyd RD, Bauman DE, Fox DG, Scanes CG. Impact of metabolism modifiers on protein accretion and protein and energy requirements of livestock. *J Anim Sci* 69 (Supp 2):56–75, 1991.
 49. Bentham J, Rodriguez-Arno J, Ross RMJ. Acquired growth hormone resistance in patients with hypercatabolism. *Horm Res* 40:87–91, 1993.