

EFFECT OF FEED RESTRICTION ON SERUM SOMATOTROPIN, INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF BINDING PROTEINS IN CYCLIC HEIFERS ACTIVELY IMMUNIZED AGAINST GROWTH HORMONE RELEASING FACTOR

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ABSTRACT

Feed restriction often increases serum somatotropin (ST) and decreases insulin-like growth factor-I (IGF-I) in ruminants; however, the mechanisms responsible for this change in ST and IGF-I are not well defined. We investigated the effects of feed restriction on serum ST, IGF-I, IGF binding proteins (IGFBP), insulin and nonesterified fatty acids (NEFA) in cyclic Angus and Charolais heifers (n=15) previously immunized against growth hormone releasing factor (GRFi) or human serum albumin (HSAi). Cows were fed a concentrate diet *ad libitum* (AL) or were restricted to 2 kg cotton seed hulls (R) for 4 d. Each heifer received each dietary treatment in a single reversal design. As anticipated, GRFi decreased ST, IGF-I and insulin (P<.05). In addition, GRFi decreased serum IGFBP-3 (P<.01), but increased IGFBP-2 (P<.01). Feed restriction resulted in an increase in serum ST in HSAi, but not in GRFi heifers. Regardless of immunization treatment, feed restriction decreased serum IGF-I and insulin, and increased NEFA (P<.01). In conclusion, the increase in serum ST levels observed during feed restriction was blocked by active immunization against GRF. However, feed restriction resulted in decreased serum IGF-I in GRFi heifers in spite of initial low levels of IGF-I (due to GRFi). Although GRFi decreased levels of IGFBP-3 and increased levels of IGFBP-2, feed restriction for 4 d did not alter serum IGFBP.

INTRODUCTION

Concentrations of somatotropin (ST) and insulin-like growth factor I (IGF-I) under normal circumstances are positively related (summarized by 1). Serum IGF-I typically increases 12 to 18 hr after administration of ST or elevation of endogenous ST via growth hormone releasing factor (GRF) (2,3,4). Several studies have demonstrated that concentrations of IGF-I typically decrease during negative energy balance in ruminants (reviewed by 5). Restriction of feed intake usually results in an increase in ST and a decrease in IGF-I in ruminants (1,5); however, nutritional status can alter serum IGF-I without altering circulating ST (6). The IGFs circulate bound to specific, high affinity binding proteins (IGFBP) which modulate their activity. Serum IGFBP have been shown to be altered by feed restriction in neonatal pigs, rats and humans (4,7,8,9); however, data in ruminants is limited (5,10).

Restriction of feed intake results in delay in puberty (initial onset of ovulation) (11,12) and postpartum resumption of reproductive activity (i.e. first ovulation after calving) (13,14). Kinder and coworkers (12) have suggested that feed restriction alters ovarian function through a decrease in pulsatile release of LH. Metabolic signal(s) through which chronic feed restriction alters LH and ovarian function have not been elucidated (15,16,17,18).

Most evidence for a direct effect of IGF-I and IGFBP in regulating ovarian function comes from *in vitro* studies. IGF-I has been shown to enhance FSH-stimulated steroid production in murine (19) and porcine (20) granulosa cells, and IGFBP have also been shown to affect the ability of IGF-I to modulate FSH actions on granulosa cells and follicular differentiation (19,20). Therefore, ST and IGF-I represent potential signals through which energy bal-

ance may alter ovarian function. To better understand the effects of IGF-I, IGFBP and ST on ovarian function in vivo, we have utilized the model of active immunization against GRF (GRFi) (18). We have previously shown that immunization of heifers against GRF at 6 mo of age decreases serum IGF-I and ST, as well as BW gain, and decreases the percentage of heifers reaching puberty by 18 mo of age (18,21). Since feed restriction and GRFi each decrease serum IGF-I and delay puberty, the objective of the present study was to compare effects of acute feed restriction on serum levels of ST, IGF-I, IGFBP, and metabolites in GRFi and control heifers.

MATERIALS AND METHODS

The present study used cyclic Angus and Charolais heifers ($n=15$, 373 ± 8 kg, 13 mo of age) that had been previously immunized against GRF-(1-29)-(Gly)₂-Cys-NH₂ conjugated to human serum albumin (GRFi, $n=8$) or human serum albumin alone (controls, HSAi, $n=7$). All heifers reached puberty by 12 mo of age and had exhibited at least one estrous cycle (21). Heifers were housed at the Butner Beef Cattle Field Laboratory (Bahama, NC) throughout the study (December, 1989 and January, 1990).

Heifers were randomly assigned within immunization group and breed into two groups. Heifers were allowed *ad libitum* access to concentrate diet (AL; 88.5% cottonseed hulls, 5% corn, 5.1% soybean meal, 1.2% dicalcium phosphate, and .2% trace mineral salt; percentage of DM) or were restricted to 2 kg cotton seed hulls for four d (R). The *ad libitum* diet was formulated to provide over 100% of NRC requirements for metabolizable energy, protein and minerals for 400 kg non-lactating heifers (22). The R diet (cottonseed hulls at 2 kg/d) was estimated to provide 5% of daily NRC recommended requirements for cycling heifers (22). A single reversal design was used such that each heifer received each dietary treatment (AL then R; or R then AL; separated by 4 d of AL feeding). Each experimental interval was as follows: Days -3 to -1, all heifers fed AL (PRE); Days 0, 1, 2 and 3, heifers fed AL or R diets (TRT); Days 4, 5 and 6, all heifers fed AL (POST). Throughout the study, heifers were housed in an open-sided building in pens (10 to 12 heifers/pen) each equipped with individual Calan gates. Water was available *ad libitum*.

Single blood samples were collected daily (0800 prior to feeding) by venipuncture from Days -3 to 6 for analysis of IGF-I, insulin, glucose and NEFA. On Day 4 of each interval, blood samples were collected via jugular cannula at 15-min intervals for 7 hr for analysis of ST while heifers were housed in individually partitioned, stationary alleys (1.8 × .7 m). Heifers were acclimated to sampling procedure for several weeks prior to initiation of the study. Overt signs of distress were not observed. Single samples collected on Day 4 (first sample of 7 hr-interval) were used for analysis of IGFBP.

Blood samples were collected into plain glass or evacuated glass tubes (Vacutainer Tube, Becton-Dickinson, Rutherford, NJ.) for analysis of ST, IGF-I, INS, and NEFA or glass tubes containing sodium fluoride (Vacutainer Tube, Becton-Dickinson, Rutherford, NJ) for analysis of glucose. Samples were stored at 4° C until centrifugation at $1,500 \times g$ for 30 minutes on the day of (GLU) or the day after (ST, IGF-I, IGFBP, INS, NEFA) collection. Serum or plasma was stored at -20° C until analyzed.

Assays. Serum ST was measured by radioimmunoassay procedures validated by Armstrong and Spears (23). Intra- and inter-assay CV for 4 assays were 9.5% and 13.0%, respectively. Assay sensitivity, defined as 90% bound, was .85 ng/ml.

Serum IGF-I was assayed using glycyglycine hydrochloride extraction procedures as described (24) with modifications (25,26). The standard source was recombinant human IGF-I (Amgen, Thousand Oaks, CA). Anti-IGF-I rabbit serum (UBK487) was obtained from Drs. L. Underwood and J. Van Wyk (Univ. North Carolina, Chapel Hill) through the National Hormone and Pituitary Program and the NIDDK. Average intra- and inter-assay coefficient of variation were 3.5 and 3.8%, respectively. Assay sensitivity was 9.5 ng/ml.

Serum INS concentrations were analyzed via the procedures of Hales and Randle (27)

with modifications (26). Average intra- and inter-assay CV were 9.1 and 11.4%, respectively, for 3 assays. Assay sensitivity was 1.7 μ U/ml. Plasma GLU was analyzed by an automated GLU oxidase method (Glucose Analyzer 2, Beckman Instruments Inc., Brea, CA.). Serum NEFA were determined via an enzymatic, colorimetric method (WAKO Chemicals USA, Inc., Dallas, TX).

Concentrations of IGFBP-2 were determined in serum as described (28). Assay sensitivity ranged from 25 to 1000 pg/tube. Intra-assay CV was 6.6%. Serum IGFBP were separated by ligand blotting as previously described (29). Briefly, sera (1.5 ul) were separated by SDS-PAGE using 12.5% polyacrylamide gels under non-reducing conditions. Proteins were transferred to nitrocellulose filters (Schleider and Schuell, Inc., Keane, NH) and probed with 125 I-IGF-I. Autoradiographs were obtained by exposure to Kodak X-Omat AR film. Molecular weight estimates were based on prestained protein standards (Amersham Corp., Arlington Heights, IL). Band intensities were quantified by scanning densitometry.

Statistical Analyses. All analyses were conducted using GLM procedures (30). Hormone and metabolite data were analyzed using a model containing treatment (GRFi vs HSAi), heifer within treatment, diet (AL vs R), diet x treatment, diet x heifer within treatment, interval (PRE, TRT or POST), and two-way interactions involving interval. Effect of treatment was tested using the heifer within treatment mean square as the error term. Effects of diet and diet x treatment were tested using the diet x heifer within treatment mean square as the error term (31).

Frequency (pulses/7 hr) and amplitude (ng/ml) of ST release were determined for individual heifers during the 7 hr sampling period. Criteria used to define a ST pulse were 1) the pulse had to occur within 30 min of the previous nadir, 2) it had to be at least 50% greater than the previous nadir and 3) the amplitude of the pulse had to be greater than the sensitivity of the assay. Basal ST was determined after deleting peaks of ST and all observations associated with an episode of ST release.

RESULTS

The main effects of GRFi on concentrations of ST, IGF-I, metabolites and INS are presented in Table 1. As anticipated, GRFi decreased ST, IGF-I, INS and GLU; however, NEFA were not affected. Concentrations of ST were decreased largely due to a paucity of episodic release of ST (Figure 1B).

TABLE 1. MAIN EFFECTS OF ACTIVE IMMUNIZATION AGAINST GROWTH HORMONE-RELEASING FACTOR (GRFi) OR HUMAN SERUM ALBUMIN (CARRIER PROTEIN, HSAi) IN BEEF HEIFERS ON CONCENTRATIONS OF SOMATOTROPIN (ST), INSULIN-LIKE GROWTH FACTOR-I (IGF-I), IGF BINDING PROTEIN-2 (IGFBP-2), INSULIN, GLUCOSE AND NONESTERIFIED FATTY ACIDS (NEFA).

Variable	Treatment	
	HSAi	GRFi
ST, ng/ml ^a	3.4 \pm .2 ^d	1.0 \pm .1
IGF-I, ng/ml ^b	109.0 \pm 3.0 ^d	41.0 \pm 3.0
IGFBP-2, ng/ml ^c	356.0 \pm 25.0 ^d	597.0 \pm 46.0
Insulin, μ U/ml ^b	14.5 \pm .6 ^d	9.7 \pm .6
Glucose, mg/100 ml ^b	59.0 \pm .5 ^d	55.0 \pm .5
NEFA, mEq/L ^b	246.0 \pm 17.0	253.0 \pm 22.0

Values are mean \pm SE.

^aMean of 29 samples per heifer collected on Day 4.

^bMean of samples collected on Day -3 to 6.

^cSingle sample collected on Day 4.

^dHSAi vs GRFi, P < .05.

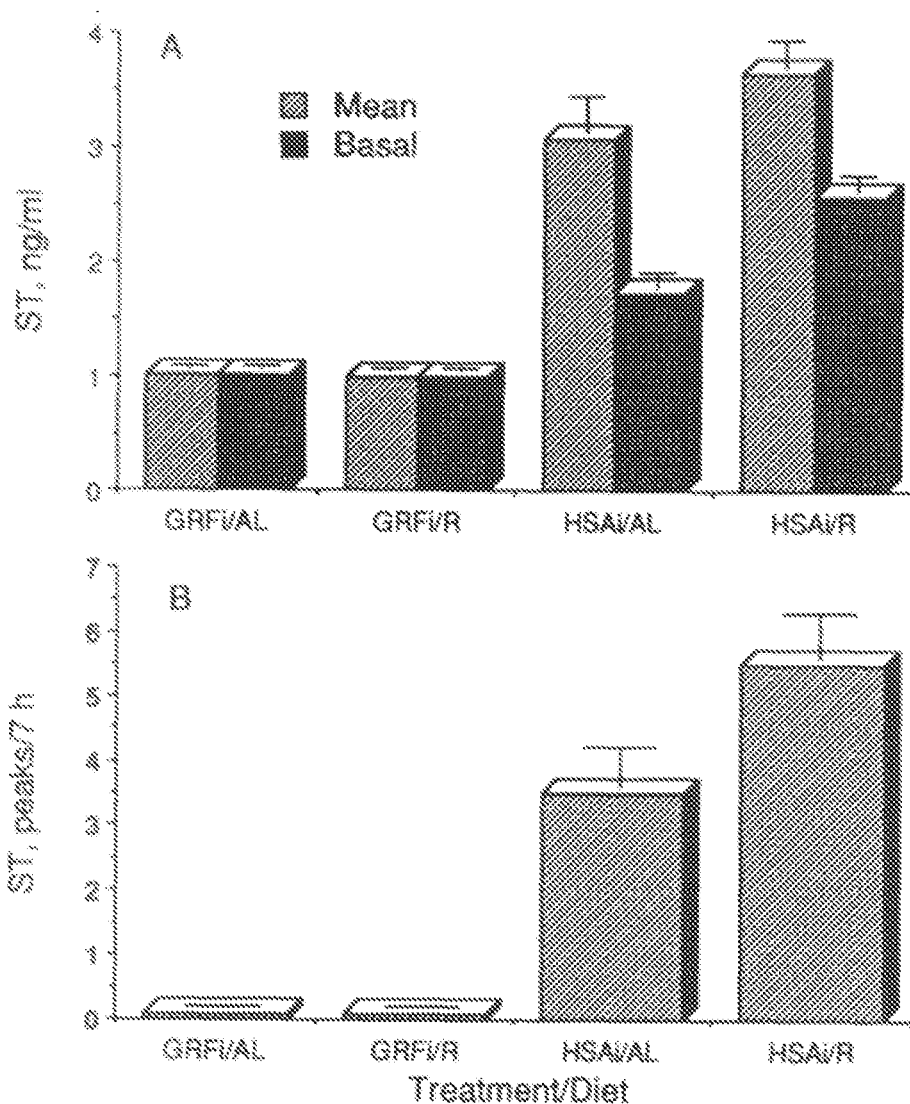


Figure 1. Mean and basal concentrations of serum ST (Panel A) and mean frequency (Panel B) of ST release (+ SE) in GRFi or HSAi heifers fed AL or R for 4 d. Samples were collected at 15 min intervals for 7 hr on Day 4.

A representative ligand blot of serum from GRFi and HSAi heifers collected on Day 4 of AL feeding is in Figure 2. Levels (arbitrary units) of IGFBP-3 (M_r 43,000 and 39,000) were greater ($P < .05$) in HSAi ($70,111 \pm 6,111$) than in GRFi ($29,676 \pm 6,247$) heifers. In contrast, serum concentrations of IGFBP-2 (determined by RIA) on Day 4 were greater in GRFi than in HSAi heifers (Table 1, Figure 3). Levels (arbitrary units) of IGFBP in serum with approximate M_r of 24,000 (presumably IGFBP-4) were similar in HSAi ($64,499 \pm 6,218$) and GRFi ($61,498 \pm 6,720$) heifers. GRFi had no effect on bands with approximate M_r of 30,000 (Figure 2).

Effects of immunization and feed restriction on mean and basal ST, as well as frequency of ST release are shown in Figure 1. Restriction of feed intake increased ($P < .05$) serum ST in HSAi heifers, with increases observed for mean and basal ST (Figure 1A), as well as frequency of episodic release (Figure 1B). As shown in Figure 4, serum ST increased 1 to 2.5 hr after feeding in HSAi/R, but not in HSAi/AL heifers. In contrast to HSAi, GRFi com-

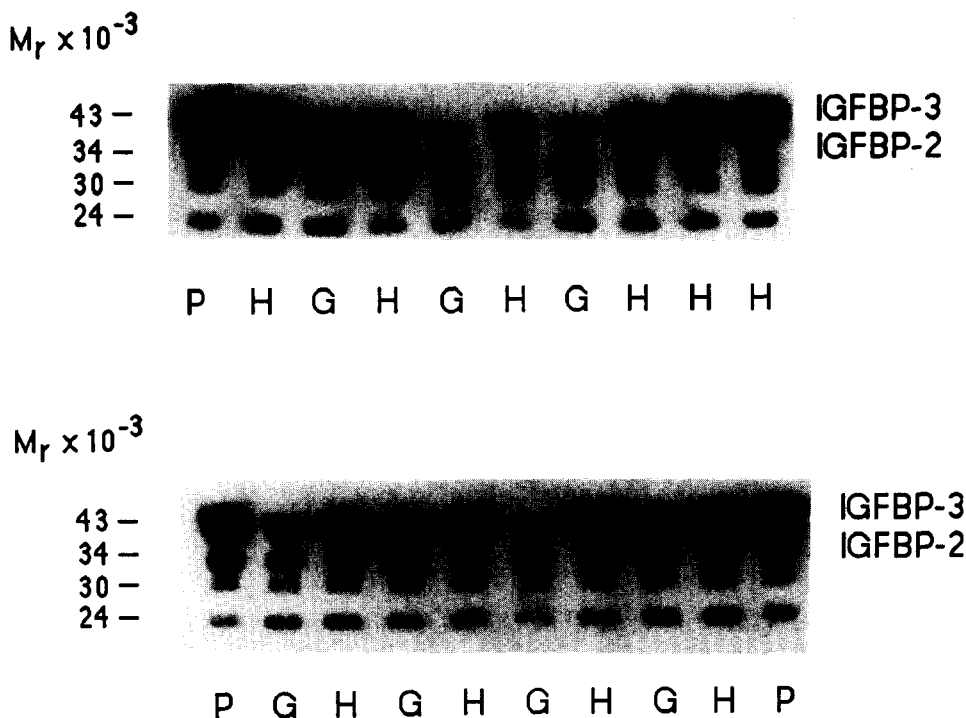


Figure 2. Forms of IGF binding proteins present in serum collected on Day 4 (single sample/heifer) from GRFi (lanes labeled G) or HSAi (lanes labeled H) heifers fed *ad libitum*. A serum pool is represented in lanes labeled P. Sera (1.5 ul) were analyzed by SDS-PAGE under non-reducing conditions on 12.5% gels and transferred to nitrocellulose filters. Filters were subjected to ligand blot analysis with ^{125}I -IGF-I and autoradiographed as described in Materials and Methods. Immunoblot analysis has confirmed the identities of bands migrating at 43,000 and 39,000 M_r as IGFBP-3 and 34,000 M_r as IGFBP-2. Relative densities of the IGFBP-3 bands were lower ($P < .01$) in GRFi versus HSAi cows, while the intensities of the 30,000 and 24,000 M_r bands were not different.

pletely blocked the effects of feed restriction on ST. Frequency of release was not different from zero, as episodic release of ST was not detected during R or AL feeding in GRFi heifers (Figure 1B).

Concentrations of IGF-I decreased during R in both GRFi and HSAi heifers (Figure 5A); a significant interval (PRE, TRT, POST) x dietary treatment interaction was detected. Similar to IGF-I, INS was lower in R than in AL in both GRFi and HSAi heifers (Figure 5B). Serum NEFA were not affected by immunization, but were significantly elevated by feed restriction (Figure 6). The treatment x diet x interval interaction was not significant for IGF-I, INS or NEFA.

A ligand blot of serum from Day 4 of AL or R feeding is depicted in Figure 7. Restriction of feed intake for 4 d had no detectable effect on relative abundance of IGFBP-3, -4 or the 30,000 M_r band. In addition, concentrations of IGFBP-2 determined by RIA were not affected by restriction of feed intake (Figure 3).

DISCUSSION

Active immunization against GRF resulted in an absence of episodic release of ST, and decreased concentrations of ST and IGF-I. This observation is consistent with previous reports in prepuberal heifers (21), cyclic (32) and lactating (33) cows, steers (34,35), lactating sows (36) and cyclic gilts (37).

This report extends our initial observations to include effects of GRFi on IGFBP. Immunoneutralization of GRF, in addition to lowering ST and IGF-I, decreased concentrations of

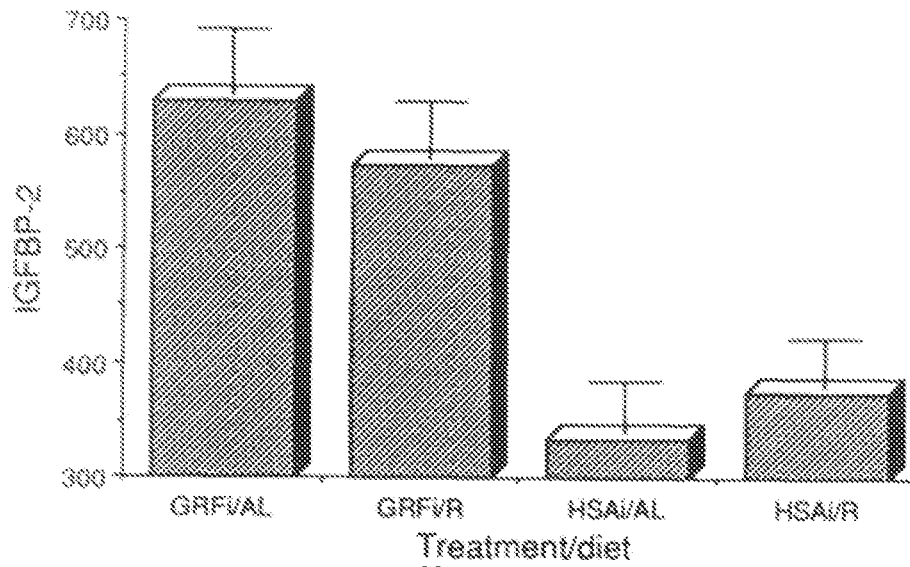


Figure 3. Concentrations of IGFBP-2 (mean + SE) determined on day 4 (last day of *ad libitum* (AL) or restricted (R) feeding). Values were determined by RIA. Serum IGFBP-2 was increased ($P < .05$) by immunization, but not altered by feed restriction.

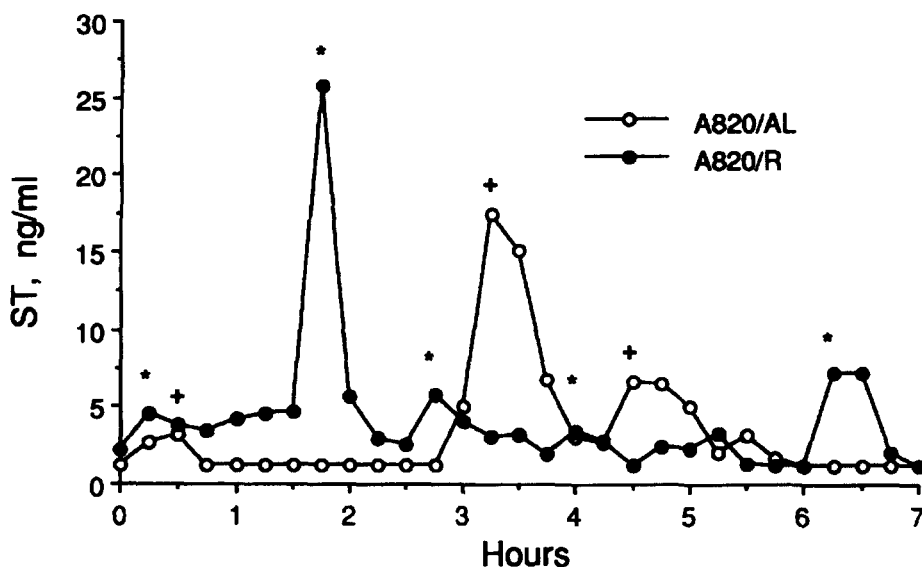


Figure 4. Profiles of serum ST from individual HSAi heifers on day 4 of AL or R feeding. Episodes of ST release are marked by '+' and '**' for AL and R intervals, respectively.

IGFBP-3 and increased concentrations of IGFBP-2. Previous reports in rodents, swine and humans have also shown a positive relationship among IGF-I, ST and IGFBP-3 and an inverse relationship among IGFBP-2 and IGF-I/ST (4,7,28,38,39). Our observation that lowering serum ST increased IGFBP-2 and decreased IGFBP-3 is consistent with observations that exogenous ST increased IGFBP-3 (28) and decreased IGFBP-2 in dairy cows (28,39).

These data provide evidence that feed restriction in ruminants elevates ST through a mechanism involving an increase in frequency of ST release. Increased ST in HSAi heifers during feed restriction was associated with an increase in frequency of ST release, whereas, feed restriction failed to alter ST in GRFi heifers. Failure of feed restriction to el-

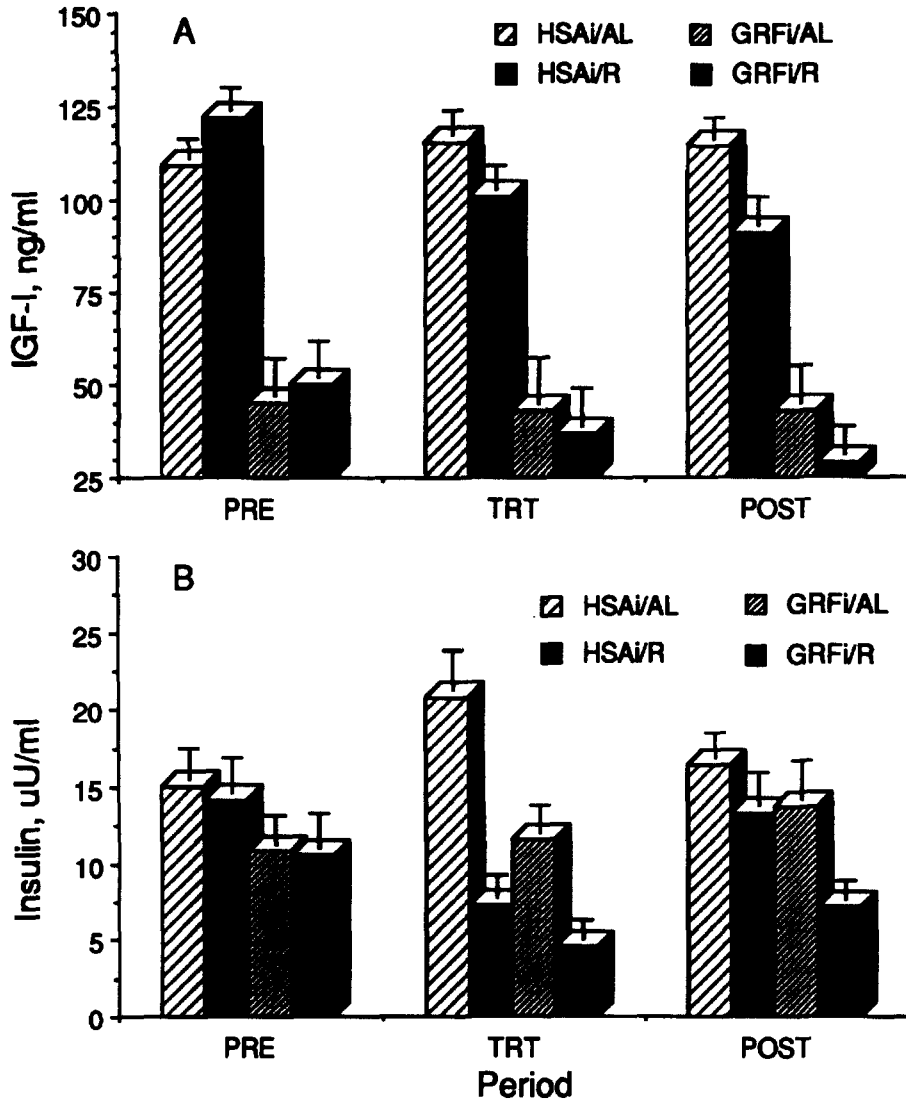


Figure 5. Concentrations (mean + SE) of serum IGF-I (Panel A) and insulin (Panel B) before (PRE, Days -3, -2, -1), during (TRT, Days 0, 1, 2, 3) and after (POST, Days 4, 5, 6) AL or R feeding in HSAi and GRFi heifers. IGF-I and insulin decreased ($P < .05$) during R in both GRFi and HSAi heifers. Across diets, insulin and IGF-I were lower ($P < .05$) in GRFi than in HSAi heifers.

evate ST via GRFi argues for a major role for GRF in the mechanism through which feed restriction increases ST. A permissive role of GRF cannot be dismissed, particularly with regard to the observation that feed restriction in wethers resulted in a decrease in hypophysal concentrations of somatostatin, but no change in portal blood concentrations of GRF (40). However, the RIA employed likely did not distinguish between immunoactive and bioactive GRF (40). Thomas et al. (40) also found that feed restriction increased amplitude, not frequency of ST release.

We hypothesized that IGF-I in GRFi heifers would not decrease during feed restriction primarily because of low stimulation by ST. In contrast, a similar magnitude of decrease in IGF-I following feed restriction was observed in GRFi and HSAi heifers (3-way interaction was not significant, $P > .3$). We believe this observation is supported by the report that the normal positive relationship between ST and IGF-I is 'uncoupled' during feed restric-

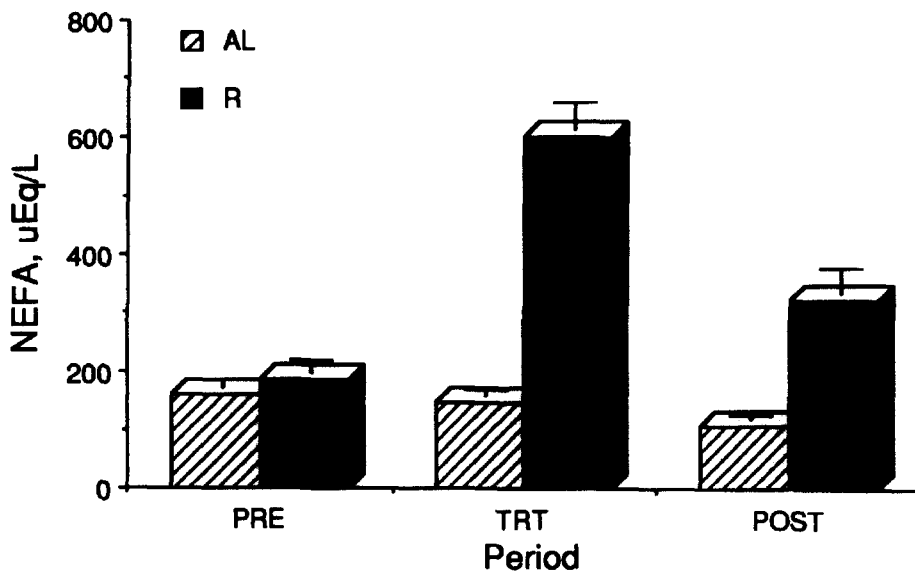


Figure 6. Mean (+ SE) NEFA concentrations in serum before (PRE, Days -3, -2, -1), during (TRT, Days 0, 1, 2, 3) and after (POST, Days 4, 5, 6) AL or R feeding in heifers. NEFA was greater ($P < .05$) in R than in AL heifers. Neither immunization or interactions involving immunization contributed to variation in NEFA, thus data were pooled across GRFi and HSAi heifers.



Figure 7. Forms of IGF binding proteins present in serum of GRFi (pairs of lanes labeled G) or HSAi (pairs of lanes labeled H) heifers collected on Day 4 of AL or R feeding (single sample per heifer). Feed restriction did not contribute to variation in intensities of bands with Mr 43,000 and 39,000 (IGFBP-3), Mr 34,000 (IGFBP-2), Mr 30,000 or Mr 24,000 (presumably IGFBP-4). IGFBP-2 measured by RIA (Figure 3) was not affected by diet.

tion, possibly involving changes in insulin or metabolites. As for IGF-I, feed restriction decreased serum INS regardless of immunization status.

In contrast to reports in humans (4,9) and pigs (7), we failed to observe an effect of acute feed restriction on IGFBP in ruminants. Other reports have failed to show a clear effect of feed restriction on IGFBP in ruminants (5); however, Vincini (39) reported that IGFBP-2 was higher during early lactation when cows were in negative energy balance. Gallaher (10) found that fasting castrate sheep decreased IGFBP-3 and increased IGFBP-2; however, fasting in pregnant ewes was associated with a slight decrease in IGFBP-3, but no change in

IGFBP-2. Although markedly affected by GRFi, both concentrations of IGFBP-3 (estimated by ligand blot) and IGFBP-2 (estimated by RIA) were not significantly altered by feed restriction. Apparently, other species of IGFBP (M_r 30,000 and 24,000) were not significantly altered by immunization or diet.

In conclusion, active immunization against GRF and the accompanying decrease in ST and IGF-I resulted in decreased serum IGFBP-3 and increased serum IGFBP-2. Feed restriction increased ST and decreased IGF-I; however, levels of IGFBP were not altered by feed restriction. Immunoneutralization of GRF blocked the effect of feed restriction on ST. In contrast, feed restriction decreased IGF-I in GRFi heifers even though IGF-I levels were already suppressed. This latter observation indicates that the additional decrement in IGF-I that occurred in feed restricted animals immunized against GRF is probably mediated through a mechanism that is independent of ST regulation.

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