EFFECTS OF RECOMBINANT DNA- DERIVED SOMATOTROPIN AND DIETARY ENERGY INTAKE ON DEVELOPMENT OF BEEF HEIFERS: II. CONCENTRATIONS OF HORMONES AND METABOLITES IN BLOOD SERA¹,²

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ABSTRACT

The effects of somatotropin (STH) and energy intake on serum concentrations of glucose (GLU), insulin (INS), nonesterified fatty acids (NEFA), urea nitrogen (UN) and insulin-like growth factor-I (IGF-I) were determined in 40 Angus heifers. At 7 mo (208 ± 8 d) of age heifers were assigned to four treatment groups: 1) vehicle (V) + high energy (HE; 2.68 Mcal ME/kg DM), 2) recombinant DNA-derived STH (20.6 mg/d; s.c.) + HE, 3) V + low energy (LE; 2.22 Mcal ME/kg DM) or 4) STH + LE. Animals remained on treatments until an average of 15.5 mo of age. Blood samples were taken every 30 min for 4 h at 9, 11, 13 and 15 mo of age to determine circulating concentrations of metabolites and hormones. Serum IGF-I was increased (P < .01) by STH injections, but this effect appeared to diminish with age (STH × age; P < .01). Energy intake did not influence IGF-I levels. Somatotropin increased (P < .01) serum GLU in heifers fed the HE diet but only tended (P = .08) to increase GLU in those fed the LE diet (STH × energy; P = .05). Although STH increased (P < .01) serum INS in both energy groups, the response in heifers fed the HE diet was greater (P < .02) than that in heifers fed the LE diet (STH × energy; P < .05). Heifers fed LE had higher (P < .01) concentrations of NEFA than heifers fed HE. Serum UN was lower (P < .01) in STH-treated heifers than for heifers treated with V. Animals fed HE had lower (P < .01) serum UN than those fed LE. These data suggest that exogenously administered STH and dietary energy may interact to influence intermediary metabolism in growing beef heifers.

(Key Words: Heifers, Somatotropin, Energy Intake, Metabolism.)


Introduction

Pituitary-derived somatotropin (STH) increases rate of body weight gain and decreases fat deposition in ruminants (Muir et al., 1983; Johnsson and Hart, 1985; Butler-Hogg and Johnsson, 1987; Sandles et al., 1987). Specifically, STH increases lipolytic activity (Hart et al., 1984) and decreases lipogenic activity (Vernon, 1982) within adipose tissue and stimulates liver secretion of insulin-like growth factor-I (IGF-I); (Schalch et al., 1979; Zapf et al., 1981), a hormone that stimulates muscle nitrogen accretion (Ballard and Francis, 1983; Froesch et al., 1986; Harper et al., 1987). Changes in growth of tissues due to STH treatment are accompanied by changes in concentrations of various hormones and metabolites. Somatotropin increased circulating levels of insulin (INS; Eisemann et al., 1986a), nonesterified fatty acids (NEFA; Peters, 1986)
and glucose (GLU; Vernon, 1982) but decreased urea nitrogen (UN; Eisemann et al., 1986b; Lough et al., 1988) in ruminants. Possible interactions between STH treatment and dietary energy have not been thoroughly investigated in growing beef heifers. Therefore, we sought to determine the effects of long-term administration of recombinant DNA-derived STH and dietary energy on circulating concentrations of hormones and metabolites in growing beef heifers.

**Materials and Methods**

*Animals and Treatments.* Animals and treatments were described previously (McShane et al., 1989). Briefly, 40 Angus heifers averaging 7 mo (208 ± 8 d) of age were allocated to four treatment groups of 10 animals each. Twenty heifers were fed a high-energy (HE) diet (2.68 Mcal ME/kg DM), whereas the remaining 20 heifers were fed a low energy (LE) diet (2.22 Mcal ME/kg DM). These diets differed only in ME and contained the same amounts of CP (12.2% on DM basis), calcium, phosphorus and vitamin A (McShane et al., 1989). Between 7 and 11.5 mo of age, each heifer received 5 kg of feed/d between 0800 and 1000. This was an adequate amount of time for all heifers to consume the total amount of feed provided each day. At 11.5 mo of age, the amount fed to each heifer was increased to 6.8 kg/d to accommodate increases in body weights. Within each feeding group, 10 heifers received daily injections of 20.6 mg STH s.c. and the remaining 10 heifers received daily injections of the sterile saline vehicle (V). Animals were maintained on treatments until an average of 15.5 mo of age.

*Experimental Protocol.* At average ages of 9, 11, 13 and 15 mo, each heifer was fitted with a jugular cannula and blood samples (10 ml) were taken at 30-min intervals between 0800 and 1000. Samples were placed on ice, allowed to clot for 24 h, and centrifuged at 1,000 x g. Sera obtained from these samples were assayed for concentrations of UN, GLU, and INS using the methods of Marsch et al. (1965), Raab and Terkildsen (1960) and Reimers et al. (1981), respectively. Serum concentrations of IGF-I and NEFA were determined in pooled samples representing each animal at each bleeding age. Serum concentrations of STH were determined for each animal at 9 and 15 mo of age. Pooled samples were prepared by combining 200 µl from each sample taken at 30-min intervals.

Serum concentrations of NEFA were determined by a modification of the radiochemical assay described by Ho (1970). A 750-µl aliquot of each serum sample was extracted with 3.5 ml of Dole's extraction mixture. N-heptane (7.5 ml) and deionized water (7.5 ml) were added, samples were vortexed, and 20 min later 3.5 ml of the upper heptane layer was transferred to 16-mm × 125-mm glass tubes containing 125 mg millic scic acid. Chloroform (3.5 ml) was added immediately. A 6-ml aliquot of each sample was transferred to 13-mm × 100-mm glass tubes and dried under air at 23°C. The dried extract was reconstituted in 2 ml chloroform-heptane (1:1) and then triplicate 250-µl aliquots were added to 400-µl microfuge tubes containing 10 µl of 65NiCl 2 in a working solution containing K 2 SO 4 , Na 2 SO 4 and triethanolamine (Ho, 1970). Tubes were vortexed for 30 s and then centrifuged for 20 min at 400 × g. A 150-µl portion of the supernatant fluid was removed and added to 5.0 ml scintillation fluid and radioactivity was determined. A standard curve of 0, 2.4, 3.2, 4.0, 5.6, 7.3, 9.7, 16.1, 22.6, 29.0 and 38.7 mg palmitic acid/100 ml N-heptane was analyzed in each assay.

*Radioimmunoassays.* Serum concentrations of INS were measured using a solid-phase RIA kit as described by Reimers et al. (1982) for use with bovine serum. The assay sensitivity was defined as the mass of insulin corresponding to 95% maximum binding of the label and averaged .55 ng/tube. Interassay and intraassay coefficients of variation were 23.3% and 8.2%, respectively. Recovery of standards (ranging from .55 to 17.5 ng/tube) from serum averaged 102.7 ± 15.6%. Inhibition curves obtained from dilutions of serum from cows were parallel to the standard curve.

Concentrations of STH were determined by an RIA validated in our laboratory (see Appendix). Serum concentrations of IGF-I were determined by a previously validated RIA (Houseknecht et al., 1988).

*Statistical Analysis.* All analyses were conducted using SAS (1985). Mean concentrations of INS, GLU and UN were determined for

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TABLE 1. EFFECT OF DIETARY ENERGY AND SOMATOTROPIN (STH) TREATMENT ON SERUM CONCENTRATIONS OF STH (NG/ML)ab

<table>
<thead>
<tr>
<th>Mean age, mo</th>
<th>High energy</th>
<th>Low energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STH Vehicle</td>
<td>STH Vehicle</td>
</tr>
<tr>
<td>9</td>
<td>173.9 41.2</td>
<td>227.1 15.2</td>
</tr>
<tr>
<td>15</td>
<td>135.3 8.2</td>
<td>124.0 11.3</td>
</tr>
</tbody>
</table>

aThe SE is 57.11 ng/ml, and each value represents the mean of 10 animals.

bTreatment effect (P < .01); age effect (P < .01); treatment × age (P = .05).

Each animal at each sequential bleeding period. The effects of energy intake, STH treatment and age and appropriate interactions on serum concentrations of IGF-I, STH, NEFA, INS, GLU and UN were determined by analysis of variance for a 2 × 2 factorial experiment with repeated measurements (Gill, 1978). The statistical model and methods for evaluating important interactions were described previously by McShane et al. (1989).

Results and Discussion

Somatotropin and IGF-I. Serum concentrations of STH in STH-treated heifers were approximately nine times greater than those in heifers treated with vehicle (Table 1). Analysis of variance revealed a STH × age interaction (P = .05) due to the fact that STH concentrations decreased (P < .05) between 9 and 15 mo of age in STH-treated heifers, but not in V-treated heifers. Serum concentrations of IGF-I also were influenced by the interaction between STH and age (P < .01). Although STH increased (P < .01) IGF-I concentrations at all ages studied (Figure 1), concentrations of IGF-I appeared to decrease with age in STH-treated animals. Dietary energy did not affect (P > .1) circulating levels of IGF-I in this experiment. However, STH and energy tended (P = .1) to interact to influence IGF-I concentrations. This may be attributed to the slightly greater elevation in IGF-I concentrations in heifers fed the HE diet compared to that in heifers fed the LE diet.

Other investigators reported that circulating concentrations of IGF-I vary with plane of nutrition (Bass et al., 1984; Clemmons and Van Wyk, 1984; Breier et al., 1986). Houseknecht et al. (1988) demonstrated that heifers deficient in dietary energy had lower circulating IGF-I concentrations than heifers on high-energy diets, despite elevated STH levels. In the above-mentioned studies, starvation and diets deficient in energy decreased IGF-I levels. In our experiment, heifers fed the LE diet were not subjected to severe energy restriction and received amounts of other nutrients that were the same as those fed the HE diet. Therefore, the modest energy restriction achieved by feeding the LE diet may have been insufficient for altering IGF-I synthesis, secretion or metabolism. Even though an effect of energy was not detected, the tendency for an interaction between STH and dietary energy on IGF-I concentrations is consistent with the concept that STH-stimulated IGF-I secretion is greater with higher energy intake.

Insulin and Glucose. Energy intake interacted (P < .05) with STH to influence serum concentrations of INS (Figure 2). Heifers fed the HE diet and treated with STH had higher (P < .02) INS concentrations than the STH-treated heifers fed the LE diet. However, concentrations of INS in heifers receiving V and fed HE were not different (P > .10) from those in heifers treated with V and fed LE. Serum concentrations of GLU (Figure 3) also were influenced by the STH × energy interaction (P = .05). Somatotropin treatment increased (P < .01) GLU concentrations in heifers fed the HE diet, but only tended (P = .08) to increase GLU in heifers fed the LE diet. In addition, concentrations of GLU in
SSTH-treated heifers fed the HE diet were higher (P < .05) than those in STH-treated heifers fed the LE diet, whereas GLU concentrations in V-treated animals were not influenced by energy.

There was a decrease in both INS and GLU levels between 9 and 11 mo of age and a decrease in GLU between 13 and 15 mo of age. These changes preceded the time at which feed supply was increased (11.5 mo) and therefore may be the results of decreased feed intake per kilogram of body weight as the heifers grew. The increase in INS and GLU concentrations between 11 and 13 mo of age occurred after feed allowance was increased (11.5 mo).

Diet affects circulating levels of INS in ruminants by altering short-chain fatty acid production in the rumen (Trenkle, 1970). Consumption of diets containing readily fermentable carbohydrates leads to an increase in propionate produced in the rumen (Luther and Trenkle, 1967) and increases INS secretion (Bassett, 1974; Hove, 1978; Gill and Hart, 1981) compared with consumption of diets low in energy and high in cellulose. In the ruminant, high circulating concentrations of INS and glucagon occur concurrently with high endogenous GLU production rates post-feeding (Bassett, 1972). Injection of propionate into the jugular vein stimulates INS secretion in ruminants (Manns and Boda, 1967) to a greater extent than injection of GLU (Trenkle, 1970). Results of these and other experiments (Trenkle, 1972; Vasilatos and Wangsness, 1980; Bines et al., 1983) suggest that volatile fatty acids, and not GLU, are the major metabolites controlling INS secretion in the ruminant. Therefore, it is not surprising that concentrations of INS were higher in heifers fed HE than in heifers fed LE, even though dietary energy level did not significantly affect concentrations of GLU in this study.

In our experiment, GLU concentrations were elevated in STH-treated heifers despite the very high circulating levels of INS. Chung et al. (1985) observed increased plasma GLU levels in pigs treated with porcine pituitary STH despite a twofold elevation in plasma INS concentrations. In addition, pituitary STH treatment resulted in increased conversion of propionate to GLU in liver slices from lactating cows (Pocius and Herbein, 1986). However, Eisemann et al. (1986a) reported high levels of INS with no change in blood GLU in heifers treated with pituitary STH for 14 d and suggested that STH treatment results in a new steady-state level of INS secretion. Increased levels of INS also were reported in lambs treated with pituitary STH (Davis et al., 1969; Wagner and Veenhuizen, 1978). Hart et
al. (1984) demonstrated that fasted wethers injected with either pituitary or recombinant DNA-derived STH for 2 d had a lower response to an INS challenge, as indicated by the ability to lower blood levels of GLU, implying an alteration in target cell sensitivity to INS. Vernon (1982) showed that ovine STH antagonizes the lipogenic action of INS in cultured sheep adipose tissue. Walton and Etherton (1986) demonstrated antagonistic effects on both pituitary and recombinant DNA-derived porcine STH on INS-stimulated GLU metabolism in swine adipose tissue. The inhibitory effect of STH on GLU uptake by adipose and other tissues, and(or) increased glucose production by the liver, may explain the elevated glucose levels observed in our experiment. Whether the elevated GLU levels or STH caused the increased INS concentrations in STH-treated heifers remains to be determined.

Nonesterified Fatty Acids. Heifers fed the LE diet had higher (P < .01) serum concentrations of NEFA (Figure 4) than did heifers fed the HE diet. Somatotropin treatment did not significantly influence NEFA levels. Concentrations of NEFA appeared to increase prior to and decrease following the increase in daily feed allowance at 11.5 mo of age. Insulin levels (Figure 3) appeared to be inversely related to NEFA concentrations.

Circulating concentrations of NEFA increase during periods of restricted intake of feed (Gill and Hart, 1981; Peters, 1986; Waghorn et al., 1987). Our results concur with these previous observations. The elevated concentrations of NEFA in heifers fed the LE diet may suggest that rate of lipolysis exceeded rate of lipogenesis (Bauman et al., 1976).

Previous studies have shown that treatment with either pituitary or recombinant DNA-derived STH elevates NEFA concentrations in the blood (Hart et al., 1984; Eisemann et al., 1986a). Although Peters (1986) reported increased blood levels of NEFA due to pituitary STH treatment, this effect was observed only in feed-restricted steers and not in steers with ad libitum access to feed. Although we did not detect a significant effect of STH on levels of NEFA, the highest concentrations of NEFA were exhibited by STH-treated heifers fed the LE diet. This observation is consistent with the concept that the effects of STH on fat mobilization may be dependent on nutritional status.

Urea Nitrogen. Heifers treated with STH had lower (P < .01) UN than did heifers treated with V (Figure 5). Decreased UN also has been reported in lambs (Davis et al., 1969) and swine (Chung et al., 1985) treated with pituitary STH. The depressed levels of UN due
to STH treatment may be attributed to increased muscle uptake of amino acids, decreased rates of mobilization of amino acids from muscle, and(or) decreased hepatic catabolism of amino acids. The effects of STH on circulating levels of UN in ruminants likely are mediated by IGF-I. Insulin-like growth factor-I has been shown to decrease protein degradation rate relative to synthesis rate in muscle cells in vitro (Janeczko and Etlinger, 1984; Ballard et al., 1986; Harper et al., 1987). Decreased protein degradation or increased protein synthesis would result in lower circulating levels of UN because fewer amino acids would be available for utilization by the liver. Eisemann et al. (1986b) reported increased nitrogen retention and decreased urinary nitrogen excretion in growing beef heifers treated with pituitary STH for 14 d. Heifers fed the HE diet had lower \( P < .01 \) levels of UN than did heifers consuming the LE diet. This may reflect a lack of energy available in the LE diet for optimal protein synthesis at the levels of dietary protein provided in the diet. Also, the effects of dietary energy on circulating levels of UN in this experiment may be due to increased recycling to the rumen, as well as to decreased absorption of ruminal ammonia in animals fed high-energy diets (Kennedy, 1980).

In conclusion, our results suggest that STH and dietary energy may interact to influence some aspects of intermediary metabolism in growing beef heifers. The effects of STH and dietary energy on adipose and muscle metabolism probably are responsible for the previously reported changes in growth and body composition (McShane et al., 1989). The mechanisms by which STH and IGF-I function at the cellular level to influence differential utilization and mobilization of GLU, NEFA and amino acids by muscle and adipose tissue in growing cattle warrants further investigation.

Appendix

Somatotropin RIA. Recombinant DNA-derived STH\(^8\) was radiolabeled with .5 mCi Na\(^{125}\)I using the chloramine-T method (Greenwood et al., 1963). The reaction was initiated by adding 30 \( \mu \)g chloramine-T to a reaction vial containing 5 \( \mu \)g STH, 20 \( \mu \)l .5 M sodium phosphate buffer (pH 7.4) and Na\(^{125}\)I. The reaction proceeded for 45 s and was terminated with 250 \( \mu \)g sodium metabisulfite. The reaction mixture was transferred to a 30-cm \( \times \) 1-cm column packed with Sephadex G-75-120\(^9\). The column was eluted with barbital-buffered saline containing 5% bovine serum albumin (BSA) and 7-ml fractions were collected into 35, 12-mm \( \times \) 75-mm borosilicate glass tubes containing 300 \( \mu \)l 5% BSA-barbital-buffered saline. Radioactivity was determined in 25-\( \mu \)l aliquots from each collection tube to characterize elution volumes representing the labeled hormone and free Na\(^{125}\)I.

Assays were run in 12-mm \( \times \) 75-mm borosilicate glass tubes. Standards were made from dilutions of STH (oGH NIADDK-I-4) ranging from .2 ng/tube to 20.0 ng/tube. One hundred-microliter aliquots of standards, control serum (pooled cow serum) or unknowns were added to appropriate tubes. The first antibody (NIADDK-anti-oGH 2; 1:200,000 final dilution) was added to all tubes, excluding tubes for determining non-specific binding (to which 100 \( \mu \)l normal rabbit serum was added). Enough .1% gel-phosphate-buffered saline solution (PBS) was added to each tube to bring the total volume to 700 \( \mu \)l. The tubes then were allowed to incubate at 4°C for 24 h. One hundred microliters (25,000 dpm) of radiolabeled STH was then added to all tubes. After a 48-h incubation at 4°C, goat antirabbit gamma globulin\(^10\) (1:500 final dilution) was added to all tubes, which then were incubated for an additional 24 h at 23°C to precipitate the first antibody. Two milliliters of PBS were added to each tube before centrifugation at 1,800 \( \times \)g for 30 min. Supernatant fluids were decanted and radioactivity of the remaining pellet was determined. The sensitivity of the assay averaged .5 ng/tube, which was the mass of STH corresponding to 95% maximum binding of the label. The interassay coefficient of variation for three assays was 18.6%. Intraassay coefficient of variation for samples averaged <10%. The recovery of unlabeled STH standards ranging from .5 ng/tube to 20.0 ng/tube averaged 94 \( \pm \) 5%. Inhibition curves observed with dilutions of pooled cow serum and selected serum samples from STH-treated

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8American Cyanamid, Princeton, NJ.
940-120 \( \mu \) beads; Sigma Chemical Co., St. Louis, MO.
10Biotek Inc., Lenexa, KS.
and V-treated heifers were parallel to the standard curve.

**Literature Cited**


