Relationship of Liver and Skeletal Muscle IGF-1 mRNA to Plasma GH Profile, Production of IGF-1 by Liver, Plasma IGF-1 Concentrations, and Growth Rates of Cattle[1] (43172)

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Abstract. **Growth hormone (GH), insulin-like growth factor-1 (IGF-1), and thyroid hormone (T3 and T4) concentrations in blood plasma of 18 crossbred cattle (six bulls, six steers, and six heifers) were measured over an 8-hr period. One week later at slaughter, IGF-1 production by liver slices and IGF-1 mRNA concentrations in skeletal muscle and liver were measured. Bulls had higher ($P < 0.05$) mean plasma GH and GH peak amplitudes ($P < 0.01$) than heifers, and values for steers were intermediate between bulls and heifers. Baseline GH concentrations and number of GH peaks were not significantly different for the three groups. Bulls had 1.6-fold ($P < 0.01$) and 3.0-fold ($P < 0.01$) greater liver IGF-1 mRNA concentrations than steers or heifers, respectively, whereas the steers had 1.8-fold ($P < 0.05$) greater IGF-1 mRNA in liver than heifers. Production of IGF-1 by liver slices was greater ($P < 0.05$) in bulls than steers or heifers. Bulls had 1.3-fold greater plasma IGF-1 than steers ($P < 0.01$), whereas steers had 1.8-fold greater plasma IGF-1 than heifers ($P < 0.01$). There were no significant differences in concentrations of skeletal muscle IGF-1 mRNA between the three groups of animals. Liver IGF-1 mRNA, liver IGF-1 production, and plasma IGF-1 were all significantly correlated with gain and mean GH peak amplitude, but not with GH baseline, GH peak frequency, or concentrations of T3 and T4. Concentrations of IGF-1 mRNA in skeletal muscle were not correlated to gain or any parameter of the GH profile. Plasma concentrations of T3 were significantly ($P < 0.05$) negatively correlated to plasma GH baseline concentrations. Muscle IGF-1 mRNA concentration was negatively related to plasma T4 and T3. The results of this study suggest that the cascade of events starting with secretion of GH from the pituitary, expression of liver IGF-1 mRNA, and secretion of IGF-1 by the liver are important phenomena for growth of cattle.** [P.S.E.B.M. 1991, Vol 196]

Growth hormone (GH) is a determinant of serum concentrations of insulin-like growth factor-1 (IGF-1), a 70-amino acid polypeptide synthesized primarily in the liver (1) and responsible for normal skeletal growth (2, 3). Abundance of IGF-1 mRNA in the liver is regulated by GH (4–6). When high doses of GH have been administered to hypophysectomized rats, IGF-1 mRNA synthesis has also been stimulated in nonhepatic tissues (5–8). This observation, along with evidence showing that arterially infused GH has local growth-promoting properties that can be abolished with antiserum to IGF-1 (9) as well as the ability of many tissues to synthesize IGF-1 (10), suggest an autocrine or paracrine role of IGF-1 for control of growth. The importance of the contribution of hepatically derived circulating IGF-1 to growth, however, cannot be disregarded. Administration of exogenous IGF-1 to hypophysectomized rats and Snell dwarf mice has resulted in stimulation of growth (3, 11).

The importance of IGF-1 synthesis in hepatic or

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nonhepatic tissues of normal, intact animals in relation to their growth potential has not been studied. Because the liver is the primary source of plasma IGF-1 (1), if there were no differences in metabolic clearance, then it seems that faster growing animals would have greater IGF-1 mRNA concentrations as well as IGF-1 synthesis in the liver. However, if IGF-1 is acting primarily in an autocrine/paracrine fashion in response to GH, and assuming gene expression is indicative of local IGF-1 production, it seems that nonhepatic tissues of faster-growing animals, specifically skeletal muscle, may have greater expression of the IGF-1 gene. If IGF-1 is acting in an autocrine/paracrine fashion in skeletal muscle, the importance of determinants of muscle growth other than GH, such as the thyroid hormones, are unknown. The purpose of this study was to compare IGF-1 mRNA concentrations in liver and skeletal muscle, liver IGF-1 production, and plasma IGF-1 concentrations of cattle with different growth rates and relate these parameters to the nature of their GH secretory profiles and thyroid status.

Materials and Methods

Animals and Experimental Procedures. Eighteen crossbred cattle, from Simmental sires and Angus × Charolais × Simmental and Hereford × Charolais × Simmental crossbred cows, were used (six as bulls, six as steers, and six as heifers). The cattle were born in the spring within a 31-day period and were maintained on pasture with their dams until weaning at 6.5 months of age. At 6 months of age, six of the males were castrated. At 8 months of age, all animals were adjusted to a diet containing on a dry basis 53.4% cracked corn, 8.0% ground cobs, 5.0% molasses, 30.0% dry corn gluten feed, and 1.18% ground limestone, 0.30% NaCl, and 2.12% of a mixture to provide recommended concentrations of vitamin A, trace minerals, and monensin sodium. Animals were housed in a common pen but individually accessed fed *ad libitum* by use of electronic gates (American Calan, Inc., Northwood, NH) until the end of the study (168 days). Animals were given fresh feed once per day, had *ad libitum* access to water, and were weighed monthly.

At 14 months of age, each animal received indwelling catheters (Tygon microbore tubing, 0.02 mm i.d. × 0.066 mm o.d.; Fisher Scientific, Pittsburgh, PA) that were inserted into the jugular vein and held in place with elastic tape. The catheters were maintained overnight by filling with sterile physiologic saline containing 100 units of heparin/ml. The next day, serial blood samples were collected into heparin at 15-min intervals for 8 hr. Blood samples were centrifuged and plasma was stored at −10°C until assayed for GH, IGF-1, total thyroid hormone (T4 and T3).

One week after blood sampling, the animals were killed at a commercial abattoir. Samples of blood, one skeletal muscle sample (Sterno-mastoid), and two samples of liver from the caudate lobe were obtained from each animal. Sections of muscle and liver weighing approximately 100–150 g were immediately placed in sterile bags, frozen in liquid nitrogen, and stored at −70°C until needed. Additional samples of liver weighing 10–15 g were placed in 250-ml Erlenmeyer flasks containing ice-cold sterile Ham's F-12 with L-glutamine (Sigma Chemical Co., St. Louis, MO) supplemented with 1% (w/v) bovine serum albumin, fraction V (Boehringer Mannheim, Indianapolis, IN), 0.06 g/liter of penicillin-G (1662 units/mg), and 0.10 g/liter of streptomycin sulfate (750 units/mg) (this mixture will be referred to simply as medium). The flasks containing the liver samples were flushed with oxygen and kept in ice for approximately 1 hr, after which the tissues were sliced for incubation studies (see below).

An estimate of muscling was calculated by measuring the ribeye area (cross-sectional area) of the longissimus dorsi at the level of the 12th and 13th ribs. An estimate of subcutaneous carcass fat was obtained by measuring the fat thickness over the 12th rib.

Isolation of Nucleic Acids. The size of IGF-1 mRNA transcripts in both the liver and skeletal muscle were analyzed by using Northern hybridizations. Quantitation of IGF-1 mRNA was achieved using a solution hybridization assay. For the Northern analyses, total RNA was extracted from each individual tissue sample by using the guanidinium isothiocyanate-cesium chloride method (12). Briefly, 0.5 g of liver or 1.0 g of muscle was homogenized in 8 ml of 4 *M* guanidinium isothiocyanate, layered on 4 ml of 5.7 *M* cesium chloride, and ultracentrifuged at 174,000*g* for 21 hr. After ultracentrifugation, the RNA pellet on the bottom of the tube was transferred to a microfuge tube and ethanol precipitated. The total RNA sample was then stored at −70°C until further use. Poly(A)$^+$ RNA was isolated by using the oligo d(T) cellulose method (13), as modified by Lund *et al.* (14). For solution hybridizations, total nucleic acid (TNA) preparations from the liver samples where obtained by the sodium dodecyl sulfate-proteinase K digestion, phenol/chloroform extraction method described by Durnam and Palmiter (15). The amount of DNA in each TNA sample was determined fluorometrically by using the method of Labarca and Paigen (16). Preparations of TNA from muscle were obtained by homogenizing the tissue in guanidinium isothiocyanate and ultracentrifugation in cesium chloride as already described, with the exception that the cesium chloride fraction was collected after centrifugation (17), ethanol precipitated, and DNA content determined as described. Muscle was done differently from liver because TNA from muscle prepared as described for liver was not satisfactory for the solution hybridization.

Hybridization Assays. Extracts of RNA from liver

and skeletal muscle were analyzed by gel electrophoresis to qualitatively characterize the species of IGF-1 mRNA present in bovine tissues. Liver poly(A)$^+$ RNA and muscle total RNA were separated by electrophoresis in a 1.5% agarose-0.66 *M* formaldehyde gel (17, 18) and transferred to Zeta Probe nylon membranes (Bio-Rad Laboratories, Richmond, CA) by capillary blotting procedures (19). Filters were prehybridized for 5 min at 65°C in 1% bovine serum albumin, 1 m*M* EDTA, 7% sodium dodecyl sulfate (SDS), and 0.5 *M* $NaPO_4$ (pH 7.2). Hybridization was performed in the same solution containing 1.0×10^6 cpm/ml of human IGF-1 cDNA (20) labeled with random priming. A gel-purified *Pst*I insert of plasmid containing the 664-base pair (bp) hIGF-1 (coding for 14 residues of the pre-pro protein; B, C, D, and E peptides; and 284 3′-UTR nucleotides) was labeled to a specific activity of approximately 1×10^8 cpm/μg by using [^{32}P]dCTP and random prime reagents obtained from Amersham (Arlington Heights, IL). Labeled probe was separated from unincorporated nucleotides by using a Sephadex G-50 (Pharmacia, Piscataway, NJ) column (0.7 × 30 cm). After an overnight hybridization, filters were washed twice at room temperature for 30 min each in 2× standard saline citrate (SSC), 0.1% SDS; twice at 65°C for 45 min each in 2× SSC, 0.1% SDS; and finally twice at 42°C for 30 min each in 0.1× SSC, 0.1% SDS. Autoradiography was performed by exposing Kodak X-Omat AR film (Eastman Kodak, Rochester, NY) to the membranes at −70°C in the presence of two intensifying screens (Du Pont Cronex Lightning Plus; Du Pont, Wilmington, DE) for up to 2 days. Transcript size was determined by comparison with RNA size standards (Bethesda Research Laboratories, Gaithersburg, MD).

Quantitation of IGF-1 mRNA was done by solution hybridization as described by Durnam and Palmiter (15) and modified by Mathews *et al.* (6). The 664-bp human IGF-1 cDNA probe (20) was subcloned into the pGEM 3Z(+) plasmid (Promega Biotech, Madison, WI) (21) in both orientations and sequenced by the Sanger dideoxy method (22). An antisense cRNA probe was synthesized according to the protocol of Melton *et al.* (23) by using [^{35}S]UTP. The labeled hIGF-1 cRNA probe was hybridized to triplicated TNA or RNA samples from each animal in 40 μl of incubation buffer containing 0.6 *M* NaCl, 20 m*M* Tris-HCl (pH 7.5), 4 m*M* EDTA, 0.1% SDS, 10 m*M* dithiothreitol, 25% formamide, and approximately 1.8×10^3 cpm of labeled cRNA probe. After an overnight incubation at 72°C, samples were treated with 40 μg of ribonuclease A (Sigma) and 250 units of ribonuclease T_1 (Boehringer Mannheim, Indianapolis, IN) in the presence of 100 μg of herring sperm DNA in a total volume of 1 ml at 37°C for 45 min. Protected probe was precipitated by using 100 μl of 6 *M* trichloroacetic acid, collected on no. 34 glass fiber filters (Schleicher and Schuell, Keene, NH), and counted in scintillation counter. The hybridization signal was compared with that of a standard curve of known amounts of a synthetic single-stranded 664-bp hIGF-1 DNA, which was synthesized from the pGEM 3Z(+) plasmid (24).

Liver Incubation. Liver samples were removed from the flasks, blotted dry, and sliced by using a Stadie-Riggs Microtome (Thomas Scientific, Swedesboro, NJ). Slices (0.5-mm thick) from each animal were weighed, rinsed in medium (composition given above), and placed into four 25-ml Erlenmeyer flasks containing 3 ml of fresh medium (40–120 mg liver/flask). Immediately after addition of the liver slices, the flasks were flushed for 15 sec with oxygen, capped, and placed in a water bath maintained at 37°C. The liver slices were incubated for 18 hr and the atmosphere above the medium was gassed with oxygen every 2–4 hr. In preliminary experiments, bovine liver slices treated in this manner continued to consume oxygen, decreasing to 20% of initial rates at 24 hr. Accumulation of IGF-1 in the medium increased with time and was increased 25–40% by the addition of bovine GH. At the conclusion of the 18-hr incubation, the medium was removed, frozen, and stored at −10°C until assayed for IGF-1.

IGF-1 Extraction. Samples of liver were cut from frozen tissue and pulverized in liquid nitrogen. The pulverized tissue was homogenized in 1 *M* acetic acid as described by D'Ercole *et al.* (10). Plasma and samples of incubation medium were acidified with addition of 1 ml of 0.5 *M* HCl to 250 μl of sample. IGF-1 was extracted from all acidified samples by using a modified method of Goddard *et al.* (25). Sep-pak C_{18} columns (Waters Associates, Milford, MA) were prewashed in order with 5 ml of isopropanol, 5 ml of high-performance liquid chromatography grade methanol, and 5 ml of 4% acetic acid. After application of the acidified sample, the column was washed with 20 ml of 4% acetic acid. Samples were eluted with 4 ml of methanol. Methanol was evaporated to dryness with a stream of air at 37°C, and the samples were reconstituted with assay buffer.

Radioimmunoassays. Plasma GH and IGF-1 concentrations were determined by using double-antibody radioimmunoassay techniques. Ovine GH, purified from ovine pituitary glands (26) and human IGF-1 (recombinantly derived; IMCERA Bioproducts, Inc., Terre Haute, IN) were iodinated by the chloramine-T method of Greenwood *et al.* (27). The labeled hormones were purified again immediately before use by exclusion chromatography using Sephadex G-150 and the buffer for each respective assay.

The GH radioimmunoassay (RIA) components were: iodinated ovine GH, reference bovine GH (28), rabbit antibovine GH (raised against bovine GH), normal rabbit serum, and goat anti-rabbit IgG (Vanguard Scientific, St. Louis, MO). The reagents were diluted in

pH 7.6 phosphate-buffered saline (0.28 *M* phosphate, 0.15 *M* NaCl, and 0.025 *M* disodium EDTA). Samples and reference GH were incubated with antibovine GH for 2 days at 4°C before addition of labeled GH. The tubes were incubated at 4°C for an additional 2 days before separation of the labeled GH bound to antibody with antirabbit IgG. Bovine thyroid-stimulating hormone, interstitial cell-stimulating hormone, prolactin, and plasma from hypophysectomized cattle were not active in this assay. Sequential dilutions of bovine plasma and extracts of bovine anterior pituitary gland paralleled the standard curve obtained with the bovine GH standard. The GH RIA had intra- and interassay coefficients of variation of 2.4 and 4.6%, respectively.

At the time of GH assay, plasma samples from times 0 to 75, 90 to 165, 180 to 255, 270 to 345, and 360 to 480 (min) were pooled for analysis of IGF-1. The IGF-1 RIA components were: iodinated human IGF-1, reference human IGF-1 (IMCERA Bioproducts Inc.), rabbit anti-human IGF-1 (Somato C22V01, raised against human IGF-1 isolated from plasma and obtained from National Hormone and Pituitary Program, Baltimore, MD), normal rabbit serum, and goat anti-rabbit IgG (Vanguard Scientific). The reagents were diluted in pH 7.5 phosphate buffer (0.03 *M* phosphate, 0.01 *M* disodium EDTA, and containing 200 mg of protamine sulfate and 200 mg sodium azide/liter). Samples and reference IGF-1 were incubated with antihuman IGF-1 for 2 days at 4°C before addition of labeled IGF-1. The tubes were incubated at 4°C for an additional 2 days before separation of the labeled IGF-1 bound to antibody with antirabbit IgG. This antiserum had 0.5% cross-reaction with IGF-1 and less than 0.1% with insulin. Extracted bovine plasma and human IGF-1 standard curves demonstrated parallelism. The IGF-1 RIA had intra- and interassay coefficients of variation of 2.8 and 9.4%, respectively.

Concentrations of plasma T4 and T3 were measured using commercial radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). The ranges of concentrations of standard were 10–240 ng/ml for T4 0.2–6 ng/ml for T3. Serial dilutions of bovine plasma were assayed with both the T4 and T3 assays and found to parallel the standard curves. Validation of the assays was also done by adding a fixed aliquot of bovine plasma to each of the standards, the results of which also paralleled the standard curve. The plasma samples were assayed at 25 and 100 μl for the T4 and T3 assays, respectively.

Statistical Analysis. Growth hormone secretory peaks were determined by a mathematical model similar to that used by Riley *et al.* (29). The mean of all values falling below the overall GH mean for the 8-hr period was calculated for each animal and was termed the inlying mean. A GH peak was at least 50% higher than the inlying mean and contained at least two points elevated above the inlying mean. The mean amplitude of the GH peaks was defined as the average of the maximum values of each of the GH secretory peaks. After identification of the peaks, all peaks and adjacent points above the inlying mean were removed from the GH profile, and the average of the remaining points was termed mean baseline GH.

Overall effects of sex and castration were analyzed as a completely random design by using analysis of variance followed by *F* and least significant difference tests (30). Correlations were calculated by using linear regression, and significance of correlation coefficients was determined by using the Fisher null hypothesis (30).

Results

Growth rates over the 168-day period and carcass measurements are reported in Table I. Bulls were shown to have the greatest daily gain and the most efficient use of feed. There were no significant differences found in daily feed intake among the three groups. Carcass weight, rib eye area, and back fat were significantly different in bulls compared with heifers. A summary of the GH profiles, IGF-1 parameters, and thyroid hormone concentrations is given in Table II. Plasma GH profiles were asynchronous for all animals and showed considerable variation between animals within each group. The mean GH concentrations were 2.3 times higher ($P < 0.05$) in bulls than in heifers. Baseline GH concentrations were not different among the three groups of animals. The number of secretory peaks was variable among individuals, and there were no significant differences. GH profiles of heifers tended to have a few small GH peaks, with a mean magnitude of 1.8 ng/ml. Steers and bulls exhibited nearly twice the number of peaks as seen in heifers as well as significantly greater ($P < 0.01$) amplitudes of GH peaks. Mean amplitude of GH peaks in bulls was 1.7 times greater ($P < 0.01$) than in steers.

Examples of Northern analysis of liver and muscle IGF-1 mRNA from two animals are shown in Fig-

Table I. Growth and Carcass Characteristics of Bulls, Steers, and Heifers ($n = 6$ of each)[a]

Parameter	Bulls	Steers	Heifers	SEM
Birth weight (kg)	40.0	43.0	41.9	2.5
Weaning weight (kg)	254.9b	245.1b	218.7c	11.4
Feedlot phase				
Daily gain (kg)	1.64b	1.45b,c	1.26c	0.13
Feed/gain (kg/kg)	6.20b	6.57b,c	7.44c	0.44
Carcass characteristics				
Carcass weight (kg)	359.6b	328.7b,c	304.1c	17.6
Loin eye area (cm^2)	95.7b	84.3c	83.6c	5.8
Backfat (cm)	0.87b	1.27c	1.25c	0.2

[a] Means within a row without a common letter differ ($P < 0.05$).

Table II. Comparison of Plasma GH Profiles and IGF-1 Parameters between Bulls, Steers, and Heifers

Parameter	Bulls	Steers	Heifers	SEM
Mean plasma GH concentration (ng/ml)	3.7b[a]	2.9b,c[a]	1.6c[a]	0.9
Mean GH baseline (ng/ml)	1.7	1.6	1.4	0.4
Mean GH peak number	4.0	4.5	2.3	1.1
Mean GH peak amplitude (ng/ml)	10.1b[a]	5.8c[a]	1.8d[a]	2.7
Liver IGF-1 mRNA abundance (amol/μg DNA)	3.29b[a]	2.02c[a]	1.11d[a]	0.29
Liver IGF-1 production (ng/g)	54.6b[a]	34.7c[a]	30.5c[a]	10.4
Mean plasma IGF-1 concentration (ng/ml)	428b[a]	327c[a]	186d[a]	66
Muscle IGF-1 mRNA abundance (amol/μg DNA)	0.089	0.059	0.071	0.010
Total T4 (ng/ml)	74.1e[b]	89.7f[b]	85.1e,f[b]	4.6
Total T3 (ng/ml)	2.1	2.5	2.2	0.58

[a] Means within a row without a common letter differ ($P < 0.05$).
[b] Means within a row without a common letter differ ($P < 0.07$).

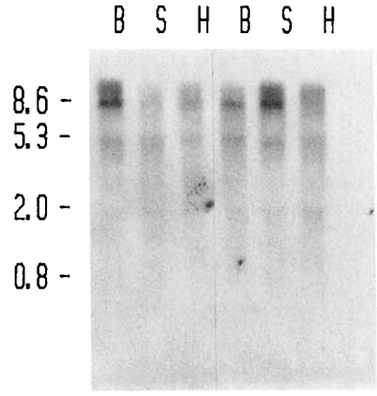

Figure 1. Samples of hybridizations of human IGF cDNA to bovine liver RNA. Approximately 20 μg of poly(A)$^+$ RNA from the liver of bull (B), steer (S), or heifer (H) were resolved on a denaturing agarose/formaldehyde gel, transferred to a nylon membrane, and hybridized to a ^{32}P-labeled human IGF-1 cDNA as described in Materials and Methods. Each lane represents the mRNA from one animal.

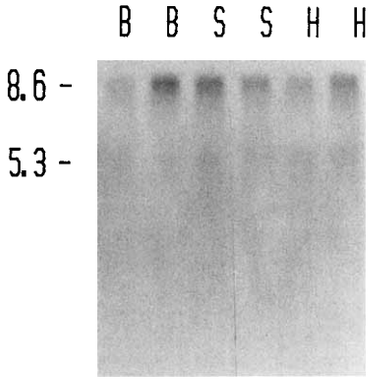

Figure 2. Samples of hybridizations of human IGF cDNA to bovine skeletal muscle RNA. Approximately 20 μg of total RNA from skeletal muscle of bull (B), steer (S), or heifer (H) were resolved on a denaturing agarose/formaldehyde gel transferred to a nylon membrane, and hybridized to a ^{32}P-labeled human IGF-1 cDNA as described in Materials and Methods. Each lane represents the RNA from one animal.

ures 1 and 2, respectively. The major IGF-1 transcripts in liver were approximately 8.0, 5.6, and 2.0 kilobases (kb). An 0.8-kb species was present in very low relative abundance. Similar IGF-1 transcripts were observed in skeletal muscle, except that 2.0- and 0.8-kb species seemed to be present in much lower relative abundance. Each of the four transcripts was present in the liver and skeletal mRNA of each animal.

Serial dilutions of a TNA sample from liver or RNA from muscle resulted in a curve paralleling that of the single-stranded hIGF-1 DNA used as standard in each solution hybridization assay. The labeled antisense IGF-1 cRNA did not hybridize to serial dilutions of yeast RNA (0–100 μg). A ^{35}S-labeled sense IGF-1 cRNA did not hybridize to serial dilutions of liver or muscle RNA. Results of the solution hybridization assays are shown in Table II. Bulls had 1.6-fold ($P < 0.01$) and 3.0-fold ($P < 0.01$) greater liver IGF-1 mRNA than did either steers or heifers, respectively. Steers had 1.8-fold greater concentration of IGF-1 mRNA in liver than did heifers ($P < 0.05$). Similar relative differences among the three groups of animals were obtained when using liver RNA slot blots hybridized to a ^{32}P-labeled phIGF-1 cDNA (data not shown).

Production of IGF-1 by liver slices was significantly greater ($P < 0.05$) for bulls than for steers or heifers. Liver content of IGF-1 seemed to be greater for bulls than for steers or heifers, but the IGF-1 in the liver seemed to be due to the concentration of hormone in residual blood in the tissue. After correction for residual plasma by reacting hemoglobin with benzidine (31), liver content of IGF-1 was not significantly different from zero ($P > 0.05$) for all three groups (data not shown). Liver slices were washed in medium before incubation to remove residual blood plasma, so it is unlikely that the production data represent leaching of IGF-1 present in tissue at the time of sampling.

Concentrations of plasma IGF-1 in bulls were 1.3 times ($P < 0.01$) that of steers and 2.3 times ($P < 0.01$) that of heifers (Table II). Plasma concentrations of IGF-1 varied little over the 8-hr period.

Although bulls had 52% greater IGF-1 mRNA in muscle than did steers, there was not a statistically significant difference in IGF-1 mRNA abundance in muscle among the three groups of animals due to variation of muscle IGF-1 mRNA in bulls. Bulls had the highest concentration of IGF-1 mRNA in muscle, whereas the heifers had the next greatest and the steers the least concentration. Similar relative differences were obtained when using muscle RNA slot blots hybridized to a ^{32}P-labeled phIGF-1 cDNA (data not shown). Bulls and steers had approximately 35-fold greater IGF-1 mRNA abundance in the liver than in the muscle, whereas heifers had only 15-fold higher concentrations of IGF-1 mRNA in the liver compared with muscle.

The steers had 20% ($P < 0.07$) and 19% greater plasma T4 and T3, respectively, than the bulls, whereas the heifers were intermediate between the steers and bulls.

Correlation coefficients for the relationship of liver IGF-1 mRNA, liver IGF-1 production, plasma IGF-1, and skeletal muscle IGF-1 mRNA with certain growth and plasma GH profile parameters are given in Table III. Average daily gain was significantly correlated to liver IGF-1 mRNA abundance, liver IGF-1 production, and plasma IGF-1 ($P < 0.01$), but not correlated to skeletal muscle IGF-1 mRNA abundance. Liver IGF-1 mRNA abundance, liver IGF-1 production, and plasma IGF-1 were significantly correlated to mean GH peak amplitude, but not to GH peak frequency or mean GH baseline. Skeletal muscle IGF-1 MRNA abundance was not correlated to any of the parameters of the GH profile. Liver IGF-1 mRNA abundance was significantly correlated to liver IGF-1 production ($P < 0.05$) as well as plasma IGF-1 ($P < 0.01$). Although liver IGF-1 production *in vitro* was not significantly correlated to IGF-1 concentrations in plasma taken 7 days before the liver samples, a significant correlation was found between liver production of IGF-1 and IGF-1 concentrations in plasma obtained from the animals at the time of death ($P < 0.01$) (data not shown). The mean concentrations of IGF-1 in these plasma samples and the differences among the three groups of animals were similar to data obtained from the blood samples taken during the longer sampling period the previous week.

The correlation coefficients for the relationship of plasma T4 and T3 with liver IGF-1 mRNA, liver IGF-1 production, plasma IGF-1, skeletal muscle IGF-1 mRNA, plasma GH profile parameters, and growth are shown in Table IV. Most of the relationships between these parameters and thyroid status were negative. Plasma T3 was negatively related to mean plasma GH, and the primary component of the GH profile it was negatively correlated to was mean GH baseline concentrations. Whereas liver IGF-1 mRNA was not correlated to plasma T4 and T3, skeletal muscle IGF-1 mRNA was negatively related to plasma T4 and T3 concentrations ($P < 0.07$).

Discussion

The males used in our study grew faster than the females before as well as after weaning. Castration of the males resulted in growth intermediate between bulls and heifers. Thickness of subcutaneous fat, cross-sectional area of longissimus dorsi muscle, and body weight gain per unit of feed indicated that bulls had more muscle and less fat than heifers. These differences are in agreement with previous comparisons of the growth of bulls, steers, and heifers, which indicated that bulls were superior to steers and heifers in rate of gain, feed utilization, and yield of lean meat (32).

GH profiles of bulls and steers were characterized as having similar baseline and frequency of periods of secretion, but greater amplitude of GH peaks as compared with heifers. Female cattle did not have higher

Table III. Correlation of Liver IGF-1 mRNA, Liver IGF-1 Production, Plasma IGF-1, and Skeletal Muscle IGF-1 mRNA with Certain Growth and Plasma GH Profile Parameters (Data Pooled from All 18 Animals)

Item	Liver IGF-1 mRNA (amol/μg DNA)	Liver IGF-1 Production (ng/g)	Plasma IGF-1 (ng/ml)	Muscle IGF-1 mRNA (amol/μg DNA)
Average daily gain (kg)	0.77 (<0.01)[a]	0.59 (<0.01)	0.67 (<0.01)	0.05
Mean plasma GH (ng/ml)	0.64 (<0.01)	0.21	0.61 (<0.01)	−0.06
Mean GH baseline (ng/ml)	0.04	0.20	0.30	−0.26
Number of GH peaks	0.40	0.13	0.45	0.00
Mean GH peak amplitude (ng/ml)	0.73 (<0.01)	0.47 (<0.05)	0.67 (<0.01)	0.19
Liver IGF-1 mRNA (amol/μg DNA)	—	0.55 (<0.05)	0.85 (<0.01)	0.13
Liver IGF-1 production (ng/g)	0.55 (<0.05)	—	0.42	−0.02

[a] Probability that correlation coefficient could not be arrived at with a pair of random, unrelated variables. No *P* value under a correlation coefficient indicates not significant, null hypothesis cannot be rejected.

Table IV. Correlation of Total Plasma T4 and T3 with Liver IGF-1 mRNA, Liver IGF-1 Production, Plasma IGF-1, Skeletal Muscle IGF-1 mRNA, Plasma GH Profile Parameteres, and Growth (Data Pooled from All 18 Animals)

Item	Plasma T4 (ng/ml)	Plasma T3 (ng/ml)
Average daily gain (kg)	−0.37	−0.04
Mean plasma GH (ng/ml)	−0.20	−0.45 (<0.06)[a]
Mean GH baseline (ng/ml)	−0.14	−0.50 (<0.05)
Number of GH peaks	−0.04	−0.35
Mean GH peak amplitude (ng/ml)	−0.29	−0.37
Liver IGF-1 mRNA (amol/μg DNA)	−0.30	−0.18
Liver IGF-1 production (ng/g)	−0.10	0.24
Plasma IGF-1 (ng/ml)	−0.37	−0.20
Skeletal muscle IGF-1 mRNA (amol/μg DNA)	−0.44 (<0.07)	−0.43 (<0.07)

[a] Probability that correlation coefficient could not be arrived at with a pair of random, unrelated variables. No *P* value under a correlation coefficient indicates not significant, null hypothesis cannot be rejected.

baseline concentrations of GH than males, as was reported for rats (33). The male pattern of GH secretion observed in bulls seemed to be partly retained in steers that had not been exposed to testicular secretions for 8 months. These animals were castrated at 6 months of age, which is prepubertal in cattle. Neonatal imprinting, by testosterone, of hypothalamic regulation of GH secretion has been suggested (34) as the reason for retention of some of the male GH characteristics after castration of rats.

The negative correlation between plasma T3 concentrations and mean plasma GH or GH baseline concentrations was not expected. Secretion of GH has been shown to be impaired during hypothyroidism in rats (35), and *in vitro*, thyroid hormones have been shown to stimulate GH gene transcription as well as GH synthesis and secretion (36, 37).

The size and number of IGF-1 mRNA transcripts found in liver of cattle were similar to those reported for rat (4, 5) and human (38) liver. The primary sequence of bovine IGF-1 is identical to human IGF-1 (39) and 96% homologous to rat IGF-1 (5).

There was a significant correlation between plasma GH and abundance of hepatic IGF-1 mRNA in these animals. The stimulatory effect of exogenous GH on liver IGF-1 mRNA has been well documented in GH-deficient rats (4–6). We have now shown in this study that the range in variation of endogenous plasma GH typically found in cattle of different sexes is related to concentration of IGF-1 mRNA in liver.

The greater concentration of IGF-1 in plasma of bulls could be from several tissues or the result of decreased metabolic clearance. The correlation of liver IGF-1 mRNA concentrations with IGF-1 production and plasma concentrations of IGF-1 suggest that a significant portion of the increased plasma IGF-1 concentrations observed in bulls resulted from increased synthesis and secretion of IGF-1 from the liver. Although they were not significant, correlations between plasma thyroid hormone concentrations and liver IGF-1 status tended to be negative and similar to the relationship between plasma thyroid hormones and plasma GH measurements.

Though overall mean GH concentration was correlated with abundance of liver IGF-1 mRNA and plasma IGF-1 concentration, all measurements of hepatic IGF-1 status of these animals were significantly related to amplitude of GH peaks rather than to baseline concentrations or number of GH peaks. The GH secretory pattern of male rates characterized as having higher amplitude than female rats is associated with faster growth (33, 34). In hypophysectomized rats, administration of GH in a pattern mimicking endogenous secretion was more effective than continuous infusion for increasing growth (40) or increasing IGF-1 mRNA concentration in skeletal muscle and rib growth plates (8). In a study with steers, augmenting endogenous secretion with exogenous GH increased nitrogen retention, but the response was not influenced by pattern of administration (41).

In contrast to liver, concentration of IGF-1 mRNA in skeletal muscle was not correlated with any parameter of the plasma GH profile. Reports of the effects of GH on expression of the IGF-1 gene in muscle are conflicting. Whereas Tollefsen *et al.* (42) reported GH had no effect on IGF-1 mRNA in differentiating mouse myoblast C2 cells, abundance of IGF-1 mRNA in skeletal muscle has been shown to be stimulated by high doses of GH in rats (5, 43). It may be that expression of IGF-1 mRNA in skeletal muscle of the cattle in this study was less sensitive to the narrower range of plasma GH concentrations as compared with muscle of rats exposed to high levels of GH. To our knowledge, the negative relationship between muscle IGF-1 mRNA and plasma T4 and T3 has not been observed previously. Because physiologic concentrations of thyroid hormones stimulate skeletal muscle proteolysis through regulation of various lysosomal enzymes including RNase (44), it is possible there is less IGF-1 mRNA degradation/turnover in the muscle of cattle with lower plasma T3 and T4 concentrations.

Studies in cattle have shown that growth of skeletal muscle is a combination of increasing cellularity (number of nuclei/muscle fiber) and an increase in cell size

(protein/DNA ratio) between the ages of 4 and 13 months (110–360 kg). Skeletal muscle growth after 13 months is limited to an increase in cell size (45). The results of this study suggest that differences in skeletal muscle growth among the animals used, which at this stage of their maturity would be primarily hypertrophy in nature, could not be attributed solely to an autocrine/paracrine property of IGF-1. Studies showing that the expression of rat skeletal muscle IGF-1 mRNA declines during postnatal development (46), that circulating plasma IGF-1 is correlated to growth rates of mini poodles (47), and that exogenous IGF-1 stimulates growth of hypophysectomized rats and dwarf mice (3, 11), along with our observations, suggest that the role of IGF-1 as an endocrine modulator of growth is important. The autocrine or paracrine effects that IGF-1 might exert on increasing cellularity or cell size in the muscle of younger cattle has not been studied.

The data from our study suggest that the episodic nature of endogenous GH secretion, especially the amplitude of the GH peaks, is important for growth. The differences in plasma IGF-1 between the three groups of animals in this study seemed to be due to liver synthesis and secretion. Whereas the IGF-1 status of the liver was related to growth, skeletal muscle IGF-1 mRNA concentrations were not related to growth or to any parameter of the GH profile.

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