Insulin-like growth factor I responses to recombinant bovine growth hormone during feed restriction in heifers

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Abstract. Insulin-like growth factor I, other hormones and blood metabolites were measured in growing heifers before, during and after a 3-day period of normal feed intake and a corresponding period of reduced feed intake. In addition, 0.1 or 0.5 mg recombinant bovine GH/kg was injected daily for 5 days during normal or during and following reduced feed intake. During reduced feed intake blood concentrations of insulin-like growth factor I, insulin, \(T_4\), \(T_3\), glucose and urea-nitrogen decreased, whereas those of non-esterified fatty acids, albumin and protein increased \((P < 0.05)\). GH, insulin-like growth factor I and insulin increased, whereas urea-nitrogen decreased in response to exogenous GH when heifers were adequately fed \((P < 0.05)\). In contrast, insulin-like growth factor I did not change during GH injections while heifers received reduced amounts of feed. Therefore, during insufficient energy and (or) protein intake, characterized by low glucose, insulin and thyroid hormone levels and increased non-esterified fatty acid concentrations, insulin-like growth factor I concentrations and responses to GH administration were markedly reduced.

Administration of growth hormone enhances daily gain, bone growth, nitrogen retention, hence protein deposition as well as feed conversion in several farm animal species (Hart & Johnsson 1986). GH mostly reduces carcass fat, although not consistently in cattle (Hart & Johnsson 1986). GH also stimulates production of insulin-like growth factor I (IGF-I) in ruminants as in other species (Gluckman et al. 1987). IGF-I is identical with somatomedin-C (Klapper et al. 1983) and is produced in the liver (Russell et al. 1985), but not exclusively. Several of the effects of GH are mediated mainly or at least in part by IGF-I (Froesch et al. 1986; Spencer 1985).

In growing or mature sheep, steers and rats insufficient energy and or protein intakes are characterized by low blood levels of IGF-I in the presence of high endogenous GH concentrations (Blum et al. 1985b; Breier et al. 1986; Elsasser et al. 1986; Underwood et al. 1986). In addition, in dairy cows at peak lactation, a period characterized by negative energy and protein balances, GH concentrations were increased, whereas IGF-I concentrations were decreased (Ronge et al. 1988). IGF-I increased less and more sluggishly during administration of recombinant bovine GH (rbGH) in high-yielding dairy cows at peak lactation compared with the dry period (Ronge & Blum, unpublished observations). This pointed to reduced sensitivity and/or responsiveness of IGF-I producing cells to rbGH under conditions in which rbGH still enhanced milk yield (Ronge & Blum, unpublished observations). In high-yielding lactating cows in early lactation, low circulating IGF-I in the presence of elevated endogenous GH, together with low blood concentrations of insulin and changes in other endocrine systems, may favour substrate supply to the mammary gland, hence milk yield. However, in
Materials and Methods

Materials, experimental design and feeding

Twelve heifers (Red Holstein X Simmental, Brown Swiss X Simmental and Brown Swiss X Schweizerisches Krausvieh), weighing 160 to 220 kg, were available. They were housed at the Federal Research Station for Animal Production Grangeneuve, Posieux, Switzerland.

Each animal went through four experimental periods: 1: normal feeding; 2: normal feeding plus rbGH administration for 5 days; 3: reduced feed intake for 3 days; and 4: reduced feed intake for 3 days plus rbGH administration for 5 days. Each period lasted for 10 days and was divided into a pre-period (of 3 days), the main-period (of 5 days during which animals received rbGH and/or reduced amounts of feed) and the post-period. Between each period animals could recover for 10 days, except for period 2 which immediately followed period 1.

Two experimental series, each with 6 heifers and separated from each other by 10 months, were performed. Heifers were injected with 0.1 or 0.5 mg rbGH/kg daily for 5 days in Experiment 1 and 2, respectively. The rbGH (lots No. 4891-122 and 5423-43-1, kindly provided by Cyanamid Int Co, Princeton, NJ), was solubilized in a solution containing 0.025 molar NaHCO₃ and 0.025 molar Na₂CO₃ (final pH 9.4) at a concentration of 10 g/l. The rbGH was administered once daily immediately prior to feeding (at 07.30 h). Injections were given iv into the jugular vein (25%) and sc in the scapular region (75%).

Animals were individually fed. Before the start of the experimental periods and during periods 1 and 2, feed was provided according to recommendations (Schneeberger & Landis 1984) to allow an average daily gain of about 750 g/day. Feed intake was adjusted according to needs before period 1 (for periods 1 and 2) and before period 3 (for periods 3 and 4). The ration consisted of corn silage (basic ration) and of a protein-rich concentrate (85% soy-bean meal in Experiment 1; 50% peanut meal and 35% soy-bean meal in Experiment 2; 15% minerals and vitamins in both experiments). Both basic ration and protein-rich concentrate were provided in restricted amounts once daily at 07.30 h. To reduce energy and protein intake in periods 3 and 4, animals received 2 kg of wheat straw/day for 3 days. Then they were realimented. Energy and protein intake and requirements were calculated on the basis of net energy and on crude and absorbable protein of the gut, according to Schneeberger & Landis (1984). Water was always available ad libitum.

Blood samples were taken between 07.15 and 07.30 h, prior to injection of rbGH and prior to feeding by jugular venipuncture, using heparinized vacutainers (Becton-Dickinson, Münchenstein, Switzerland). In Experiment 1 blood samples were taken only on day 0 and day 3, in Experiment 2 as shown in figures. In addition, in period 2, blood samples were taken immediately before and at 30 min, 1, 2, 4, 8 and 24 h after the administration of 0.1 and 0.5 mg rbGH/kg.

Laboratory methods

Feed analyses (dry matter, crude protein, crude ash, crude fibre) were performed according to the Weende procedure at the Federal Research Station for Animal Production Grangeneuve, Posieux, Switzerland.

Glucose, protein, albumin, urea-nitrogen (urea-N), non-esterified fatty acids (NEFA), IRI, T₄ and T₃ in blood plasma were measured as described by Blum et al. (1985a).

Concentrations of GH were determined by radioimmunoassay according to Hart et al. (1975) with some modifications. The GH for standards and for iodination were based on hypophyseal extracts (lots No. USDA-bGH-B-1-AFP-5200 and USDA-bGH-I-1 AFP 6500; obtained from National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Baltimore, MD). Antisera against bovine rbGH (lot 5/15.6.86; kindly provided by Prof Dr D. Schams, Institute of Physiological, Technical University of Munich, Freising/Weihenstephan, FRG) was raised in a rabbit. Goat-anti-rabbit-gamma-globulin (fraction P₁; obtained from Antibodies Inc, Davis, CA) was used as second antibody (together with 5% polycethylene-glycol) to separate bound from free hormone. The GH was labelled using the iodogen procedure and the reaction was stopped with potassium iodide (10 g/l). The rbGH paralleled bGH standards obtained from hypophyseal extracts. Half-maximal binding was attained with 10 μg GH/l and the sensitivity was below 1 μg/l. Recovery of rbGH for 1 and 10 ng added (10 μl) to 1 ml of bovine serum was 117 ± 17% and 107 ± 6%, respectively. All samples from one animal were measured within the same assay and each sample was determined in triplicate. Intra- and inter-assay coefficients of variation were below 10%.

Insulin-like growth factor 1 was determined in triplicate by radioimmunoassay according to Zapf et al. (1981)
Table 1.

Bodyweight, dry matter (DM) intake of basic ration (BR), concentrates (C), net energy (NE) and crude and absorbable protein (CP and AP, respectively) during the preperiod (0) and changes from preperiod to day 3 (Δ3).

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Periodes</strong></td>
<td>1 + 2</td>
<td>3 + 4</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>192 ± 7</td>
<td>212 ± 8</td>
</tr>
<tr>
<td><strong>BR-intake (kg DM/day)</strong></td>
<td>2.56 ± .08</td>
<td>2.78 ± .06</td>
</tr>
<tr>
<td>Δ3</td>
<td>+0 ± 0</td>
<td>−1.02 ± .06</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td><strong>C-intake (kg DM/day)</strong></td>
<td>1.36 ± 0</td>
<td>1.36 ± 0</td>
</tr>
<tr>
<td>Δ3</td>
<td>+0 ± 0</td>
<td>−1.36 ± 0</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td><strong>NE-intake (MJ/day)</strong></td>
<td>26.8 ± .6</td>
<td>28.0 ± .4</td>
</tr>
<tr>
<td>Δ3</td>
<td>+0 ± 0</td>
<td>−22.4 ± .4</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td><strong>CP-intake (g/day)</strong></td>
<td>661 ± 13</td>
<td>709 ± 9</td>
</tr>
<tr>
<td>Δ3</td>
<td>+0 ± 0</td>
<td>−659 ± 9</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td><strong>AP-intake (g/day)</strong></td>
<td>469 ± 6</td>
<td>500 ± 5</td>
</tr>
<tr>
<td>Δ3</td>
<td>+0 ± 0</td>
<td>−424 ± 5</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± s.e.m. Significance of differences (Δ) of values on day 3 from values on day 0: *, P < 0.05; NS, P > 0.05.

with some modifications. Purified human IGF-I (preparation I/3 and I/4; kindly provided by Prof Dr E. Humbel, Institute of Biochemistry, University of Zürich, Zürich, Switzerland), which has the same structure as bovine IGF-I, was used for standards. For production of antiserum (in a rabbit) and for iodination (by the chloramin T method) we have used recombinant human IGF-I (rIGF-I; preparation Mu 14 Fr 25–32 TOP, kindly provided by Prof Dr J. Nüsch and Dr K. Scheibli, Ciba-Geigy AG, Basle, Switzerland). All samples were pretreated with acid/ethanol to separate IGF-I from its binding protein(s), as described by Daughaday et al. (1980). The samples were neutralized with NH₄HCO₃, lyophilized and reconstituted in the assay buffer (1 l containing 8 g NaCl, 0.2 g KCl, 1.15 g Na₂HPO₄ × 2 H₂O, 0.2 g KH₂PO₄, and 2 g of IGF-I-free bovine albumin) before further use. After incubation for 24 h with antibody and another 24 h with tracer, antibody-bound and free IGF-I were separated after addition of 1% bovine gammaglobulin (dissolved in the assay buffer) and 25% polyethylene-glycol (dissolved in bidistilled water) by centrifugation. Specific binding was between 30–40%. Half-maximal binding was 1.0 ng/tube. The sensitivity was below 0.1 ng/tube (less than 6.5 μg/l). Recovery was 91 ± 5 and 111 ± 9%, respectively, for 10 and 20 ng rIGF-I (10 μl) added to 1 ml bovine plasma. ¹²⁵I-labelled rIGF-I added to bovine plasma was recovered with 104 ± 1%. Compared with separation of IGF-I bound to plasma protein(s) by chromatography using a large column (30 × 2 cm, volume 92.5 ml) and acidified buffer, recovery by acid/ethanol extraction was 27 ± 5% higher. Diluted sera from cattle in different physiological states paralleled the standard curve. Hepar in plasma did not modify the results. The intra- and inter-assay coefficients of variation were below 15%. Recombinant human insulin-like growth factor II (lot CGP 35126, Los 810188; kindly provided by Dr W. Märki and Dr K. Scheibli, Ciba-Geigy AG, Basle, Switzerland) and serum-extracted bovine insulin-like growth factor II
Table 2.
Concentrations of hormones and metabolites in heifers during the preperiod (0) and changes on day 3 from preperiod (Δ3) in the absence and presence of recombinant bovine growth hormone (rbGH).

<table>
<thead>
<tr>
<th>Period</th>
<th>IGF-I (μg/l)</th>
<th>GH (μg/l)</th>
<th>IRI (μg/l)</th>
<th>T₃ (nmol/l)</th>
<th>T₄ (nmol/l)</th>
<th>Glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
<td>Experiment 1</td>
<td>Experiment 2</td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td>Feed intake + rbGH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal 0.1 mg/kg</td>
<td>Normal 0.5 mg/kg</td>
<td>Reduced 0.1 mg/kg</td>
<td>Reduced</td>
<td>Normal</td>
<td>Reduced</td>
</tr>
<tr>
<td>0</td>
<td>60.1 ± 2.4</td>
<td>51.9 ± 1.3</td>
<td>55.8 ± 4.0</td>
<td>46.5 ± 4.3</td>
<td>55.2 ± 7.3</td>
<td>63.5 ± 9.7</td>
</tr>
<tr>
<td>Δ3</td>
<td>+1.7 ± 1.7</td>
<td>+14.0 ± 3.3</td>
<td>-17.3 ± 4.6</td>
<td>-3 ± 6.4</td>
<td>+3.8 ± 5.6</td>
<td>+39.0 ± 16.4</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>0</td>
<td>7.2 ± 2.0</td>
<td>7.8 ± 1.4</td>
<td>8.9 ± 1.7</td>
<td>10.5 ± 1.6</td>
<td>21.2 ± 5.9</td>
<td>14.4 ± 2.3</td>
</tr>
<tr>
<td>Δ3</td>
<td>-1.8 ± 2.1</td>
<td>+14.2 ± 2.2</td>
<td>+2.5 ± 1.6</td>
<td>-2.4 ± 2.0</td>
<td>+10.8 ± 9.9</td>
<td>+39.8 ± 7.1</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>0</td>
<td>22.0 ± 0.2</td>
<td>17.0 ± 0.5</td>
<td>17.0 ± 0.3</td>
<td>11.0 ± 0.3</td>
<td>35.0 ± 0.4</td>
<td>33.0 ± 0.3</td>
</tr>
<tr>
<td>Δ3</td>
<td>-0.7 ± 0.2</td>
<td>+14.0 ± 0.7</td>
<td>-14.0 ± 0.2</td>
<td>-10.0 ± 0.3</td>
<td>-0.2 ± 0.5</td>
<td>+43.0 ± 14</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>0</td>
<td>4.42 ± 0.19</td>
<td>4.48 ± 0.27</td>
<td>4.71 ± 0.28</td>
<td>4.16 ± 0.37</td>
<td>4.10 ± 0.25</td>
<td>4.16 ± 0.12</td>
</tr>
<tr>
<td>Δ3</td>
<td>-0.22 ± 0.15</td>
<td>+21.0 ± 0.6</td>
<td>-2.0 ± 0.20</td>
<td>-1.8 ± 0.42</td>
<td>+0.0 ± 0.22</td>
<td>-0.65 ± 0.35</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>0</td>
<td>92.2 ± 9.2</td>
<td>114.8 ± 10.5</td>
<td>105.3 ± 9.7</td>
<td>111.1 ± 13.3</td>
<td>122.9 ± 12.9</td>
<td>136.2 ± 9.7</td>
</tr>
<tr>
<td>Δ3</td>
<td>-12.7 ± 8.2</td>
<td>+12.7 ± 7.5</td>
<td>-30.0 ± 2.9</td>
<td>-26.8 ± 7.9</td>
<td>+3.9 ± 7.4</td>
<td>-18.2 ± 6.0</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>0</td>
<td>5.07 ± 0.06</td>
<td>5.21 ± 0.14</td>
<td>5.34 ± 0.10</td>
<td>4.74 ± 0.12</td>
<td>5.53 ± 0.14</td>
<td>5.52 ± 0.10</td>
</tr>
<tr>
<td>Δ3</td>
<td>+0.0 ± 0.05</td>
<td>+0.9 ± 0.12</td>
<td>-0.50 ± 0.05</td>
<td>-0.41 ± 0.08</td>
<td>-0.08 ± 0.07</td>
<td>+0.11 ± 0.21</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Statistical analysis

Values are expressed as means ± SEM. The significance of differences (on day 3 in Experiment 1; on day 1, 2, 3, 4 and 7 in Experiment 2) from basal levels (day 0) within each of the four experimental periods was tested separately for Experiment 1 and 2 by Wilcoxon signed rank test, whereas comparisons between Experiment 1 and 2 of basal levels or of changes (on day 3) from basal levels (on day 0) were based on Wilcoxon test, as described by Ciba-Geigy A G (1980), using the programmes of MSI Widas (Dr Wälti A G, 9470 Buchs, Switzerland).

Results

Dry matter, energy and protein intake (Table 1)

Body weights did not change significantly from periods 1 to 2 and from periods 3 to 4 (not shown), but increased from periods 1 + 2 to periods 3 + 4 in both experiments ($P < 0.05$). The dry matter intake of basic ration and concentrates, energy and protein was decreased during reduced feed intake ($P < 0.05$), whereas there were no changes during the control period and during rbGH administration (periods 1 and 2). There was an increase in dry matter, net energy and protein intake from periods 1 + 2 to periods 3 + 4 because of the increased body weight ($P < 0.05$). Intake of crude protein was higher, whereas intake of absorbable protein was lower in Experiment 2 than 1. The decrease of net energy and protein intake was comparable in both experiments.

Hormones (Table 2, Fig. 1)

Concentrations of IGF-I did not change significantly in normally fed controls throughout period 1. After the administration of rbGH during period 2, concentrations of IGF-I remained unaffected for the first 3 h (data not shown), but increased after 1 day ($P < 0.05$) and then remained elevated up to the end of the experimental period ($P < 0.05$). Concentrations of IGF-I in controls increased more when 0.5 mg than 0.1 mg rbGH/kg was administered, but not significantly. In period 3, concentrations of IGF-I decreased within 48 h during reduced feed intake ($P < 0.05$), then returned to basal concentrations within 1 day of refeeding. Concentrations of IGF-I did not change signifi-
santly in limited fed heifers injected with rbGH during period 4.

Basal concentrations of GH were higher in Experiment 2 than 1, but not significantly. Concentrations did not change significantly in period 1. In period 2, after injection of 0.1 and 0.5 mg rbGH/kg, respectively, concentrations of GH transiently increased above basal levels, at 30 min by 65 ± 15 and 2480 ± 469 µg/l; at 1 h by 86 ± 21 and 947 ± 151 µg/l; at 2 h by 56 ± 10 and 730 ± 173 µg/l; at 4 h by 50 ± 8 and 364 ± 69 µg/l; at 8 h by 22 ± 6 and 216 ± 32 µg/l; and at 24 h by 3 ± 7 and 47 ± 18 µg/l (data not shown). Concentrations of GH did not change significantly during the reduced feed intake of period 3. In response to rbGH administration during the reduced feed intake of period 4, concentrations of GH increased even more than during period 2 when animals were adequately fed, but not significantly.

Basal concentrations of IRI were on the average higher in Experiment 2 than in Experiment 1 (P < 0.05). During period 1, concentrations of IRI did not change significantly. Concentrations of IRI increased above basal values within 1 day and remained elevated as long as rbGH was administered, but the increase was significant only in period 2 when 0.5 mg rbGH/kg was given (P < 0.05). In period 3, concentrations of IRI decreased within 24 h during reduced feeding (P < 0.05) and then returned to basal concentra-

![Fig. 1.](image)

Blood concentrations (means ± SEM) of insulin-like growth factor I (IGF-I), growth hormone (GH), insulin (IRI), triiodothyronine (T₃) and thyroxine (T₄) during periods 1, 2, 3 and 4 of Experiment 2. Heifers were injected 0.5 mg recombinant bovine growth hormone (rbGH)/kg daily for 5 days in periods 2 and 4 (†). Feed intake was reduced for 3 days during periods 3 and 4 (■). Significance of difference from mean basal levels: * P < 0.05; no symbol: P > 0.05.
tions within 1 day during refeeding. In period 4, IRI also decreased during reduced feed intake combined with administration of 0.1 mg rbGH/kg ($P<0.05$). However, the decrease was smaller than in period 3 and no longer significant if 0.5 mg rbGH/kg was administered in restrictively fed animals. Concentrations of IRI markedly increased above basal levels within 1 day of refeeding combined with rbGH administration ($P<0.05$).

Concentrations of T3 and T4 during period 1 did not change significantly. In period 2, concentrations of T3 did not change significantly, whereas T4 slightly decreased in heifers receiving 0.5 mg rbGH/kg ($P<0.05$). During reduced feed intake (periods 3 and 4) both T3 and T4 decreased within 2 to 3 days and to a similar extent in the absence as well as in the presence of rbGH ($P<0.05$). During re-alimentation both T3 and T4 returned within 1 to 2 days to basal levels.

Metabolites (Table 2, Fig. 2)
Glucose did not change significantly in periods 1 and 2 in the absence and presence of rbGH, but in periods 3 and 4 the level decreased within 1 day during reduced feed intake ($P<0.05$). The decrease was smaller during reduced feed intake in the presence than in the absence of rbGH, but not significantly.

Concentrations of NEFA did not change significantly in periods 1 and 2 above basal levels during rbGH administration (but not higher than values in period 1). NEFA markedly increased within 1 day during reduced feed intake in periods 3 and 4 ($P<0.05$), and tran-

![Graph of Metabolites](image.png)

**Glucose**

- Period 1: 6.0 mmol/l
- Period 2: 5.0 mmol/l
- Period 3: 4.0 mmol/l
- Period 4: 3.0 mmol/l

**NEFA**

- Period 1: 0.6 mmol/l
- Period 2: 1.3 mmol/l
- Period 3: 1.5 mmol/l
- Period 4: 2.0 mmol/l

**Urea-N**

- Period 1: 3.0 mmol/l
- Period 2: 4.0 mmol/l
- Period 3: 5.0 mmol/l
- Period 4: 6.0 mmol/l

**Albumin**

- Period 1: 20 g/l
- Period 2: 20 g/l
- Period 3: 20 g/l
- Period 4: 20 g/l

**Protein**

- Period 1: 60 g/l
- Period 2: 60 g/l
- Period 3: 60 g/l
- Period 4: 60 g/l

**Days**

Blood concentrations (means ± SEM) of glucose, non-esterified fatty acids (NEFA), plasma urea-nitrogen (urea-N), albumin and protein during periods 1, 2, 3 and 4 in Experiment 2. Heifers were injected 0.5 mg recombinant bovine growth hormone (rbGH)/kg daily for 5 days in periods 2 and 4 (●). Feed intake was reduced for 3 days during periods 3 and 4 (□). Significance of difference from mean basal levels: *, $P<0.05$; no symbol, $P>0.05$. 

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siently fell below basal levels within 1 day of refeeding \((P < 0.05)\).

Basal concentrations of urea-N were significantly higher in Experiment 2 than 1 \((P < 0.05)\). Concentrations of urea-N did not change significantly in period 1, but slightly decreased during rbGH administration in period 2 \((P < 0.05)\) and more with 0.5 than with 0.1 mg rbGH \((P < 0.051)\). In periods 3 and 4, concentrations of urea-N markedly decreased within 1 day during reduced feed intake in the absence and in the presence of rbGH \((P < 0.05)\).

Albumin and protein did not change significantly during period 1. Albumin decreased in period 2 during treatment with 0.5 mg rbGH/kg \((P < 0.05)\). Both albumin and protein increased during reduced feed intake in the absence and presence of rbGH (periods 3 and 4), although only significantly in Experiment 2 \((P < 0.05)\).

**Discussion**

If animals were fed according to requirements, concentrations of metabolites, GH, IRI, \(T_3\) and \(T_4\) during the control period before the start of the experiments were in the range previously described for growing steers (Blum et al. 1985b; Verde & Trenkle 1987). Concentrations of IGF-I were higher than we have found in lactating cows during the first weeks after parturition and in newborn calves, but lower than in bulls towards the end of fattening and in 3-year-old bulls used for artificial insemination (Ronge et al. 1988; Ronge & Blum 1988, 1989, unpublished observations).

During period 2, when heifers were still fed according to requirements, the administration of rbGH was followed by an increase in GH, IGF-I and IRI, which was greater with 0.5 than 0.1 mg rbGH/kg, although the raise for IRI was only significant with 0.5 rbGH/kg. Similarly, IGF-I and IRI increased after GH injections in lactating cows and growing pigs (Davis et al. 1987; Etherton et al. 1987). Because it took more than 3 h before IGF-I increased after the first rbGH injection and at least 2 days before IGF-I reached maximal concentrations, this effect is barely the result only of enhanced release, but likely also the consequence of enhanced IGF-I production induced by rbGH. An increase of circulating IRI after GH injections has been observed in other studies, particularly in early periods of the administration and if high amounts were injected (Bauman et al. 1985). Insulin resistance develops in the presence of high circulating GH concentrations (Brockman & Laarveld 1986; Etherton & Walton 1986; Hart & Johnsson 1986) and this is generally thought to be the reason for the (compensatory) increase in IRI secretion. Insulin resistance can contribute to enhanced fat mobilisation and glucose production or reduced utilization of glucose during rbGH treatment. However, in our experiments, when animals were fed according to recommendations, glucose concentrations did not change and NEFA increased only slightly during rbGH treatment. The decrease of urea-N concentrations during rbGH administration can be interpreted as an expression of reduced amino acid oxidation, hence improvement of nitrogen conservation, which is a key effect of GH. Reasons for the slight but significant decrease of albumin concentrations if high amounts of rbGH were administered may be the consequence of alterations in nitrogen distribution.

Concentrations of IGF-I decreased in period 3 during reduced feed intake, as described in humans, rats, steers and sheep (Breier et al. 1986; Gluckman et al. 1987; Underwood et al. 1986). The decrease was observed already within 1 day and was maximal after 3 days. The time course of changes of IGF-I was very similar to that observed for IRI, \(T_3\), \(T_4\), glucose and urea-N, whereas IGF-I was inversely related to NEFA. Concentrations of IRI, \(T_3\), \(T_4\), glucose, NEFA and urea-N have already been shown to be markedly influenced by reduced feed, energy and protein intake in growing and lactating cattle (Hart et al. 1978; Blum et al. 1985a, b; Kunz et al. 1985). The decrease of urea-N concentrations during feed restriction was in contrast to an earlier study in which urea-N increased within 1 to 2 days during fasting (Blum & Kunz 1981). Concentrations of GH increases in growing cattle during reduced energy intake (Blum et al. 1985b; Breier et al. 1986), but in cattle it usually takes several days before a rise becomes significant. Therefore, it is not surprising that GH was not significantly changed during feed restriction for only 3 days in the present study. An elevation of protein and albumin has previously been described during fasting in steers as an expression of hemoconcentration (Blum & Kunz 1981).
In period 4, if 0.5 mg rbGH was administered during reduced feed intake, blood GH tended to increase to higher levels than when animals were fed according to recommendations, possibly due to reduced clearance from the circulation (Trenkle 1978). Concentrations of IGF-I did not increase above basal concentrations if rbGH was administered to animals receiving reduced amounts of feed, even with the higher dose. To our knowledge, reduced responsiveness to exogenous GH has not been reported in species other than man (Merimée et al. 1982). Several causes can be advanced for this effect. Reduced GH binding to the liver has been demonstrated in starving rats as a consequence of reduction in receptor number or receptor affinity for the hormone (Maes et al. 1984) and this was possibly also the case during feed intake in our heifers. Levels of IRI and responses to rbGH, as well as concentrations of T₃, T₄ were markedly decreased in our limited fed heifers. These endocrine alterations were therefore possibly also responsible for the lowered responsiveness of IGF-I producing cells to rbGH. Thus, in diabetes mellitus and hypothyroidism IGF-I production is reduced (Amiel et al. 1984). Insufficient availability of energy, expressed by reduced circulating energy-yielding metabolites, such as glucose in this study, may also impair IGF-I production. Reduced feed intake was of short duration in this study and it may be argued that this was not relevant for a long-term feed deprivation. However, we have found decreased IGF-I levels and responses to rbGH in lactating dairy cows following a period of negative energy blances for 3 to 4 weeks (Ronge & Blum, unpublished observations).

In conclusion, the present study demonstrates that in limited fed heifers, the insufficient supply of energy and protein is followed by reduction of blood levels of IGF-I, paralleled by decreasing IRI, T₃ and T₄ and, in particular, by reduced IGF-I responses to rbGH within 3 days. This demonstrates at least partial decoupling of IGF-I from GH, as suggested from studies in high-yielding dairy cows during early lactation, characterized by markedly negative energy and protein balances (Ronge et al. 1988, Ronge & Blum, unpublished observations).

Acknowledgments

This study has in part been supported by the Swiss Society of Artificial Insemination.

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Received November 28th, 1988.
Accepted February 27th, 1989.

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