Insulin-like growth factors and their binding proteins in plasma and milk after growth hormone-stimulated galactopoiesis in normally lactating women

Bernhard H Breier1, Stella R Milsom1, Werner F Blum2, Jürg Schwander1, Brian W Gallaher1 and Peter D Gluckman1

Research Centre for Developmental Medicine and Biology1, Department of Paediatrics, University of Auckland, Auckland, New Zealand; University Children’s Hospital2, Tübingen, Germany; Kantonsspital1, Basel, Switzerland


We performed a double-blind randomized placebo-controlled trial of recombinant human growth hormone (hGH) in normally lactating women (N=8 per group) to investigate the endocrine mode of action of the galactopoietic effect of this hormone. Insulin-like growth factors I (IGF-I) and II (IGF-II) and their binding proteins (IGFBP-1, IGFBP-2 and IGFBP-3) were measured by radioimmunoassay in plasma and milk samples collected throughout the study. All assays were validated for human plasma and milk. Human GH treatment (0.1 IU·kg−1·body wt·day−1 for 7 days) increased plasma concentrations of IGF-I from 22.1 ± 1.3 nmol/l (mean ± SEM) to 59.7 ± 2.5 nmol/l (p<0.01). At the end of the study the increase in plasma IGF-I correlated significantly with the increase in milk volume (r = 0.67, p<0.005, N = 16). The IGF-I levels were considerably lower in milk, with 0.14 ± 0.03 nmol/l before and 0.31 ± 0.04 nmol/l after hGH treatment. The increase in milk IGF-I levels (134.0 ± 14.5%) with hGH treatment was significant (p<0.01) and plasma and milk IGF-I concentrations correlated significantly when considering all samples of the study (r = 0.45, p<0.001, N = 56). The concentrations of IGF-II were not changed significantly with hGH treatment in plasma (52.5 ± 2.5 nmol/l before and 42.6 ± 3.9 nmol/l after treatment) or milk (2.1 ± 0.29 nmol/l before and 2.3 ± 0.49 nmol/l after hGH treatment). The IGFBP-1 levels were not changed with hGH treatment in plasma (approximately 1.3 nmol/l) or milk (approximately 0.2 nmol/l). Although IGFBP-2 concentrations in plasma were reduced significantly (p<0.05) after hGH treatment (11.1 ± 1.5 before and 8.4 ± 0.9 nmol/l after hGH treatment), milk IGFBP-2 levels did not respond to hGH treatment. Milk levels were markedly higher (sevenfold) in comparison to plasma levels. Plasma IGFBP-3 showed a delayed and smaller rise with hGH treatment in comparison to the rise observed in IGF-I. However, at the end of the study the response (38.6 ± 4.9%) to hGH was significant (p<0.01) and a significant correlation was observed also between the increase in IGFBP-3 and the increase in milk volume (r = 0.55, p = 0.03, N = 16). Plasma IGF-I and IGFBP-3 concentrations correlated significantly when considering all samples of the study (r = 0.61, p<0.001, N = 63). Milk IGFBP-3 levels were approximately 100 lower in comparison to plasma levels and did not correlate with any other measurements. Our data show that hGH-stimulated galactopoiesis in normally lactating women is mediated by significant elevations of plasma and milk IGF-I and plasma IGFBP-3. While IGF-I may be a principal mediator of the galactopoietic effect of hGH, we cannot simply attribute the action of hGH solely to a systemic rise in IGF-I. The increase in plasma IGFBP-3 with hGH treatment suggests that IGFBP-3 could facilitate the delivery of IGF-I to the mammary gland. The high concentrations of IGFBP-2 in milk suggest that mammary epithelial IGFBP-2 may direct regional tissue distribution of IGF-I to the site of milk synthesis.

Bernhard Breier, Research Centre for Developmental Medicine and Biology, Department of Paediatrics, University of Auckland, Private Bag 92019, Auckland, New Zealand

The relationship between plasma concentrations of growth hormone (GH) and galactopoiesis has been well documented in farm animals and a large body of data provides evidence that treatment of healthy dairy cows with bovine GH (bGH) increases milk yield by 10–40% (1). In addition we have shown recently that human GH (hGH) treatment increases milk volume in normally lactating women (2). Although the precise mode of action of GH-enhanced galactopoiesis is not known, a range of physiological parameters are influenced by GH treatment leading to increased cardiac output and mammary blood flow, repartitioning of absorbed nutrients and a reduction of fat deposition such that glucose and amino acids are made available for
increased synthesis of milk components (1). As functional GH receptors appear to be lacking in lactating mammary tissue, it has been suggested that the galactopoietic effect of GH may be mediated by circulating insulin-like growth factors (IGFs) or their binding proteins (IGFBPs) (1).

The IGFs are GH-dependent mitogens that are structurally and functionally related to insulin and mediate many of the biological effects of GH in an endocrine, paracrine or autocrine fashion (3). In plasma and other extracellular fluids IGFs are bound to a family of structurally related IGFBPs (4) that carry and modulate the actions of the IGFs. The majority of IGFs in postnatal plasma are bound to the GH-dependent 150-kDa ternary complex of IGFBP-3. The binding subunit of IGFBP-3 has a predicted molecular weight of 28.5 kDa (5). IGFBP-2 is a non-glycosylated protein with a predicted molecular weight of 32 kDa; it is a predominant IGFBP in fetal plasma (6) and in human milk (7). IGFBP-1, which is a non-glycosylated protein with a predicted molecular weight of 25 kDa, is GH-independent and is most prominently characterized by its inverse relationship to plasma insulin concentrations (8).

A role for IGF-I and IGFBPs in mediating the galactopoietic effect of GH has been suggested by a number of observations. Treatment with GH increases plasma concentrations of IGF-I and IGFBP-3 (4, 9). In vitro, IGF-I stimulates bovine mammary cell replication and DNA synthesis (10) and this action is likely to be via type I IGF receptors, which are present in high concentrations in mammary tissue (11). Synthesis and secretion of IGFBPs has been reported in human and animal mammary tissue (12, 13). Each of the IGFBPs could have a unique role as a modulator of IGF action and the different IGFBPs appear to be under independent endocrine and metabolic regulation (4, 9), such that GH treatment may lead to coordinated changes in the production of IGFBPs to initiate tissue-specific targeting and distribution of IGFs.

Although GH can affect mamogenesis and lactogenesis (1), which are distinct physiological events of the lactation cycle, we have investigated the role of GH treatment in normally lactating women during established lactation and consider the mode of action of this galactopoietic hormone. If the galactopoietic effect of GH is mediated by IGF-I, then mechanisms must be available that increase either IGF-I transport across the capillary endothelium or local intramammary production of IGF-I or IGFBPs. We have shown recently that treatment of normally lactating women with recombinant human GH (rhGH) increases galactopoiesis at mid-lactation (2). We now report data on IGF-I and IGF-II as well as IGFBPs (IGFBP-1, IGFBP-2 and IGFBP-3) in plasma and milk from the same study using specific radioimmunoassays. Characterization of IGFs and the IGFBPs in plasma and milk after hGH treatment provides further insight into the endocrine mechanism of GH-enhanced galactopoiesis. A preliminary abstract of the present report has been presented previously (14).

Materials and methods

Subjects

Healthy female volunteers were recruited from the community in late pregnancy or early lactation as described before (2). Subjects were aged between 18 and 40 years. were delivered of a healthy term infant with no feeding difficulties, were lactating normally, were between 8 and 18 weeks postpartum at the time of the study and were on no medication.

Study design

A randomized, double-blind placebo-controlled trial was performed with eight women per group. During the 10-day study period breast milk was collected daily at the end of the early morning feed and venous blood was obtained on days 1, 4, 6 and 10. Human GH (Genotrophin, Kabi-Pharmacia, Stockholm, Sweden) in a dose of 0.1 IU·kg⁻¹ body wt·day⁻¹ or placebo was given subcutaneously by an injection pen (Kabipen, Kabi-Pharmacia, Stockholm, Sweden) at 09.00 h on days 3–9 inclusive. Milk production was assessed on day 2 (control day) and day 9 (after 7 days of treatment) using the World Health Organization 24-h test weigh protocol (2). All studies and procedures were carried out in the subject's own home. The treatment code was not broken until completion of the study.

Ethical considerations

Approval for the trial was given by the Auckland Hospital Ethics Committee and the Standing Committee on Therapeutic Trials, New Zealand Department of Health. Volunteers and their spouses gave written and informed consent to the trial at least 2 weeks before commencement.

Radioimmunoassays

IGF-I RIA. The IGF-I concentrations in plasma and milk were measured by a double-antibody radioimmunoassay (RIA) after acid–ethanol cryo-precipitation extraction as described elsewhere (15). The primary antiserum (no. 878/4) was used at a final dilution of 1:250 000. The cross-reactivity of the antibody with IGF-II and insulin was <0.05% and <0.001%, respectively, and the minimal detectable dose of the assay was 0.07 ng/tube. Recombinant human IGF-I (batch CPG 35'126, provided by Drs K Mueller and W Maerky, Ciba-Geigy Ltd., Basel, Switzerland) was iodinated by the chloramine T method as described before (15). Plasma IGF-I values from the present study analysed by this method have been reported previously (2). We discovered during
our validation procedure for milk samples that both the classical acid–ethanol extraction as well as the modified acid–ethanol cryo precipitation failed to show parallel displacement to the standard curve (16). Major interference is caused in the RIA for IGF-I by high concentrations of residual IGFBP-2, which cannot be removed sufficiently by acid–ethanol extraction (16). As our antiserum (878/4) has low cross-reactivity with IGF-II (<0.05%), we assessed whether an analogous approach to that of Blum et al. 1988 (17) for the IGF-II immunoassay could be used for the IGF-I assay. Milk samples were extracted by acid–ethanol cryo precipitation and preincubated in assay buffer containing IGF-I antiserum (878/4) at a final dilution of 1:250,000 and human recombinant IGF-II (batch 099EM9, Eli Lilly and Co., Indianapolis, IN) (25 ng/tube) for 18–20 h at 4°C. 125I-labelled IGF-I tracer (25,000 cpm) was added and the assays were incubated for 18–20 h at 4°C. All other assay steps were identical to the method described before (15). The recovery of IGF-I added to the milk samples before extraction was 89±9.5% (N=8) and complete parallel displacement was observed between extracted samples and the standard curve. The correlation between milk IGF-I values obtained with this method and values obtained after Sephadex G-75 extraction under acidic conditions was: r=0.82, p<0.0001, slope=1.16, intercept=0.10. N=28. The intra-assay variation was 7% and the interassay variation was 10%. The advantages of this method are: high sample throughput, reliability and elimination of interference by IGFBPs in the assay. This allowed us to analyse all milk samples collected throughout the study.

All plasma samples from the present study were analysed after acid–ethanol cryo precipitation using the addition of excess IGF-II in the assay buffer as described above as part of the validation of this methodology for plasma samples. Parallelism was observed in the assay between serial dilutions of extracted plasma and the standard curve. A high correlation (r=0.94, p<0.0001, slope=0.90, intercept=7.4. N=64) was observed with values obtained when no IGF-II was added to the assay. The recovery of IGF-I added to the plasma samples before extraction was 88±4.5%. N=12. The intra-assay variation was 4% and the interassay variation was 7%. Plasma and milk IGF-I values are expressed in terms of the international reference preparation (IRR IGF-I, batch 87/518).

IGF-II RIA. The antibody was developed against the synthetic C-domain of hIGF-II (hIGF-II 33–40) as described previously (17). It was of high specificity, showing a cross-reactivity with hIGF-I of less than 0.05%. Tracer and standards were prepared from recombinant hIGF-II (kind gift of Kabi Pharmacia, Stockholm, Sweden). Radioiodination by the chloramine T method was performed as described before (17). Plasma samples were extracted by acid–ethanol according to Daughaday et al. (18). The extract was diluted 1:30 with assay buffer (0.02 mol/l sodium phosphate, pH 7.4, 0.12 mol/l NaCl, 0.2% BSA, 0.02% NaN3, 0.1% Triton X-100) before measurement. Milk samples were diluted 1:5 directly with assay buffer without extraction. Preliminary studies showed that extraction did not improve the accuracy of the measurement but reduced the sensitivity of the assay. The assay mixture was composed of 100 μl each of standard or sample, primary antibody (1:10,000) and tracer (20,000 cpm). To block the interference of IGFBPs in the assay, an excess of recombinant hIGF-I was added concomitantly with the antibody solution (25 ng/tube) before adding the tracer as described previously (17). After incubation at 4°C for 2 days, bound and unbound tracer were separated by a second antibody (17). Parallelism between the standard curve and serial dilutions of both plasma extracts and milk samples was excellent. Spiking experiments with rhIGF-II gave a recovery of 91.1±7.6%. Half-maximal displacement occurred at 0.48 ng/tube and the intra-and interassay coefficients of variation were 3.6% and 12.2%, respectively.

IGFBP-1 RIA. The antibody was produced in rabbits against hIGFBP-1 (pp12 from Dr Hans Bohn, Behringwerke, Marburg, Germany) (19) and was used at a final dilution of 1:7500. No cross-reaction with hIGFBP-2 or hIGFBP-3 was observed up to 1000 ng/tube in RIA or with other IGFBPs in western blotting. Standards and tracer were prepared from hIGFBP-1. Radiolabelling was performed as described previously (20) and the assay protocol was identical to that of the IGF-II RIA. Plasma and milk samples were diluted 1:8 and 1:4, respectively, before measurement. Good parallelism was obtained between IGFBP-1 standard curves and dilutions of both plasma and milk samples. Half-maximal displacement occurred at 2.0 ng/tube and intra- and interassay coefficients of variation were 3.4% and 8.1%, respectively.

IGFBP-2 RIA. The IGFBP-2 levels in plasma and milk were measured by a double-antibody method. The polyclonal antibody was obtained by injecting rabbits with pure recombinant human IGFBP-2 (21). Pure IGFBP-2 was used as standard and as tracer iodinated by iodogen (Pierce no. 28600) (21). The assay was performed as follows. All dilutions of serum and milk were made in phosphate-buffered saline (pH 7.2) and 3% IGFBP-free human serum albumin (Swiss Red Cross). Samples, 1 ng of tracer and the antiserum in a final dilution of 1:30,000 were incubated at 4°C for 3 days, when binding reached equilibrium. The complex was then immunoprecipitated by adding 1 unit of goat anti-rabbit immunoglobulin (Calbiochem no. 539845) and 40 μg of rabbit gamma globulin (Sigma no G-0261). After a 30-min incubation at room temperature the samples were precipitated by adding 1 ml of 6% polyethylene glycol 6000 (Serva no. 33137) and 1% Nonidet P40 (Sigma no. N-3516) and centrifuged for 10 min at 3000 g. The supernatant was then aspirated and
discarded. All samples were measured in two to three dilutions in triplicate. Dilution curves for serum and milk were parallel to the standard dilution in this assay. Intra- and interassay variations for serum were 7.4% and 8.0% (N = 55) and for milk were 8.0% and 8.5% (N = 8), respectively. The recovery of IGFBP-2 in serum was 91.2 ± 3.1% and in milk was 89.4 ± 5.2% (N = 6). There was no cross-reactivity with pure recombinant IGFBP-3 and 1–3% cross-reactivity with a purified fraction of IGFBP-1.

IGFBP-3 RIA. IGFBP-3 was measured by RIA directed against the acid-stable IGFBP-3 binding subunit as described previously (20). No cross-reaction with hIGFBP-1 or hIGFBP-2 was observed in RIA or with other IGFBPs in western blotting experiments. Plasma samples were diluted with assay buffer 1:600 before measurement, while milk samples were diluted 1:30. Validation experiments showed excellent parallelism between purified hIGFBP-3, which was used for standards and serial dilutions of plasma and milk samples.

Ligand and western blotting

IGFBPs were analysed by ligand blotting as described previously (6). Briefly, plasma samples (2 μl) were electrophoresed on 12% polyacrylamide gels and transferred to nitrocellulose. After blocking with 1% BSA, the blot was incubated with 125I-labelled rhIGF-II (150 000 cpm/ml) overnight and then washed. The nitrocellulose was exposed to Kodak X-OMAT AR diagnostic film to detect the IGFBP bands. Molecular sizes of the bands were estimated by calibration with Amersham 14C-labelled molecular weight markers. The bands were identified by comparison with purified human IGFBP-1, -2 and -3 (19–21).

For IGFBP-2 Western blotting, plasma samples were run on polyacrylamide gel electrophoresis (PAGE) gels and transferred to nitrocellulose as described above. Materials for Western blotting were obtained from Biorad Laboratories, Richmond, CA. The method is published elsewhere (7,21). In brief, the membranes were blocked using 1% BSA, incubated with IGFBP-2 antibody (1:250 dilution) overnight, washed and then incubated with alkaline phosphatase-labelled second antibody for 90 min. After washing, bound second antibody was detected with NBT/BCIP colour developer.

Results

IGF-I levels

The rhGH treatment increased plasma concentrations of IGF-I (Fig. 1) with a significant (p < 0.01) rise within 24 h of the first injection. A plateau of plasma IGF-I levels was reached after 3 days of rhGH treatment. The increase of plasma IGF-I in the rhGH-treated group was highly significant (p < 0.001), reaching 175.7 ± 19.9% compared with the pre-treatment values. At the end of the study the increase in plasma IGF-I was correlated significantly with the increase in milk volume (r = 0.67, p < 0.005, N = 16).

The IGF-I concentrations in milk were between 100- and 200-fold lower in comparison to plasma levels (Fig. 1). There was a small rise in milk IGF-I within the first 24 h of rhGH treatment, which was only significant.
IGF-II levels

There was no consistent change in plasma or milk IGF-II concentrations with rhGH treatment (Tables 1 and 2). The IGF-II concentrations in milk were between 20- and 40-fold lower than the corresponding samples in plasma. At the end of the study a significant inverse correlation \((r = -0.61, p < 0.02, N = 16)\) was observed between plasma concentrations of IGF-I and IGF-II.

IGFBP-1 levels

Plasma concentrations of IGFBP-1 did not change with hGH treatment (Tables 1 and 2). Milk IGFBP-1 concentrations were generally between six- and tenfold lower than the corresponding plasma values (Table 1). A small decrease in milk IGFBP-1 was observed by hGH treatment, which just reached statistical significance \((p < 0.05)\) at days 4 and 10 of the experiment.

IGFBP-2 levels

Plasma concentrations of IGFBP-2 decreased slightly with hGH treatment (Table 1). A significant \((p < 0.01)\) decrease could be detected only by paired comparison with pretreatment values of the rhGH group at days 4 and 6 of the experiment. At the end of hGH treatment (day 10) there was a significant \((p < 0.05)\) reduction in plasma IGFBP-2 in comparison with the placebo group. The change \((p < 0.01)\) in plasma IGFBP-2 at the end of hGH treatment was \(-20.4 \pm 8.1\%\) in comparison to pretreatment levels. At the end of the study, plasma concentrations of IGFBP-2 showed a negative correlation with plasma IGF-I values \((r = -0.56, p < 0.03, N = 16)\); a similar relationship was observed when all samples from the study were considered \((r = -0.37, p < 0.003, N = 63)\). Plasma concentrations of IGFBP-1 correlated significantly with plasma IGFBP-2 levels when taking all samples of the study into consideration \((r = 0.34, p < 0.006, N = 63)\). Milk concentrations of IGFBP-2 (Table 2) were between five- and tenfold higher in comparison to plasma levels. These surprisingly high concentrations of IGFBP-2 in milk were found to be intact IGFBP-2 with a molecular weight of 32 kDa using immunoblotting after SDS-PAGE. In addition, ligand blot analysis showed that the IGFBP-2 in milk is fully able to bind IGF-I and IGF-II suggesting that the IGFBP-2 in milk is biologically active. Although there was no significant change in milk IGFBP-2 levels with hGH treatment, the very high concentrations of this binding protein in milk indicate that milk contains a massive excess of free IGF-binding capacity.

IGFBP-3 levels

There was a small rise \((p < 0.05)\) by paired comparison with pretreatment values in plasma IGFBP-3 concentrations within the first 24 h of hGH treatment (Fig. 3).
Table 2. Milk insulin-like growth factor II (IGF-II) and IGF binding proteins (IGFBPs) in human growth hormone (hGH)-treated lactating women.

<table>
<thead>
<tr>
<th></th>
<th>IGF-II</th>
<th>IGFBP-1</th>
<th>IGFBP-2</th>
<th>IGFBP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 placebo</td>
<td>1.5 ± 0.08</td>
<td>0.22 ± 0.04</td>
<td>52.6 ± 3.6</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>Day 1 hGH</td>
<td>2.1 ± 0.29</td>
<td>0.23 ± 0.06</td>
<td>63.4 ± 7.7</td>
<td>1.5 ± 0.25</td>
</tr>
<tr>
<td>Day 4 placebo</td>
<td>1.7 ± 0.37</td>
<td>0.24 ± 0.05</td>
<td>61.3 ± 4.3</td>
<td>1.0 ± 0.18</td>
</tr>
<tr>
<td>Day 4 hGH</td>
<td>2.4 ± 0.46</td>
<td>0.12 ± 0.01*</td>
<td>82.9 ± 2.6</td>
<td>1.6 ± 0.35</td>
</tr>
<tr>
<td>Day 6 placebo</td>
<td>1.3 ± 0.08</td>
<td>0.21 ± 0.06</td>
<td>58.6 ± 6.2</td>
<td>1.1 ± 0.27</td>
</tr>
<tr>
<td>Day 6 hGH</td>
<td>2.4 ± 0.44</td>
<td>0.12 ± 0.02</td>
<td>78.1 ± 11.8</td>
<td>1.5 ± 0.22</td>
</tr>
<tr>
<td>Day 10 placebo</td>
<td>1.6 ± 0.09</td>
<td>0.20 ± 0.03</td>
<td>56.1 ± 4.7</td>
<td>1.2 ± 0.24</td>
</tr>
<tr>
<td>Day 10 hGH</td>
<td>2.3 ± 0.49</td>
<td>0.11 ± 0.04*</td>
<td>66.2 ± 15.2</td>
<td>1.8 ± 0.23</td>
</tr>
<tr>
<td>% Change placebo</td>
<td>11.4 ± 7.4</td>
<td>-1.9 ± 10.6</td>
<td>7.4 ± 7.7</td>
<td>15.1 ± 10.1</td>
</tr>
<tr>
<td>% Change hGH</td>
<td>-1.9 ± 12.3</td>
<td>-42.5 ± 10.3</td>
<td>-2.5 ± 10.6</td>
<td>30.3 ± 10.3</td>
</tr>
</tbody>
</table>

* Values are means ± SEM (nmol/l); *p < 0.05, ANOVA and Scheffé test.

Fig. 3. Plasma concentrations of insulin-like growth factor binding protein 3 (IGFBP-3) during human growth hormone (hGH) treatment in normally lactating women. Women received either hGH treatment (●) (0.1 IU·kg⁻¹·day⁻¹) or placebo treatment (♀) from day 3 until day 9. Values are means ± SEM; *p < 0.01 compared to placebo group, ANOVA and Scheffé test: a: p < 0.05 and b: p < 0.01, paired t-test compared to day 1 of study.

Fig. 4. Relationship between plasma insulin-like growth factor I (IGF-I) and plasma IGF binding protein 3 (IGFBP-3) of all samples collected during the study (r = 0.61, p < 0.001).

Discussion

Mammary gland function includes three distinct physiological states, beginning with differentiation and growth of the gland (mammogenesis) during puberty and pregnancy, the initiation of lactation (lactogenesis) at parturition and maintenance of established lactation (galactopoiesis) after parturition. The endocrine regulation of each of these physiological states is largely independent. In human lactation, for example, PRL is a most important hormone for lactogenesis, while changes in milk volume are independent of plasma concentrations of PRL during galactopoiesis (22), such that PRL plays only a permissive role in galactopoiesis. The galactopoietic effect of GH treatment is well established for a range of species, including humans (1, 2). While the physiological changes with GH-enhanced lactational performance have been well described, the endocrine mediation of these events has not been established (1).

Our present study suggests that IGF-I may be a key...
mediator of the galactopoietic effect of GH. Plasma concentrations of IGF-I were increased rapidly with hGH treatment and this increase correlated with the response in milk volume. While the IGF-I concentrations in milk were about 100-fold lower than plasma levels, there was a significant increase with hGH treatment. Using our IGF-I RIA, validated extensively for human and bovine milk, we observed (BH Breier, unpubl. data) a similar increase in milk IGF-I levels in dairy cows after treatment with bGH (0.29 ± 0.04 nmol/l before and 0.88 ± 0.16 nmol/l after bGH treatment, N = 10). While commercial pasteurized bovine milk contained 0.44 ± 0.03 nmol/l IGF-I (N = 8), markedly higher IGF-I levels were observed in bovine day-1 colostrum (10.2 ± 3.6 nmol/l, N = 5). Human day 1 colostrum and human saliva contained 0.85 ± 0.36 nmol/l (N = 5) and 0.10 ± 0.02 nmol/l (N = 5) IGF-I, respectively (BH Breier, unpubl. data). These data show that milk from hGH-treated women contains IGF-I concentrations that are well within the range of IGF-I values commonly observed in bovine milk, human colostrum and human saliva.

The highly significant correlation between milk and plasma IGF-I concentrations from our study suggests the possibility of trans-cellular passage of IGF-I through capillary endothelium of mammary tissue. As mRNA levels for IGF-I are absent or very low in rat and human mammary tissue (12, 13), and bovine mammary epithelial cells do not synthesize IGF-I (23), maternal plasma is the most likely source of IGF-I in milk. Direct evidence for vectorial transfer of IGF-I from blood plasma into milk has been shown recently in lactating goats (24). When 125I-labelled IGF-I was infused into the pudic artery of one mammary gland of goats, intact 125I-labelled IGF-I was found in milk of the infused gland at a transfer rate that could explain most, if not all, milk IGF-I to have originated from the blood plasma pool. In addition, the transfer of blood-derived IGF-I into mammary epithelium has been shown in lactating sheep using iv infusion of N-Met rhIGF-I and specific antibodies for immunohistochemical localization (25). However, it is uncertain whether short-term infusion of IGF-I into the pudic artery of lactating goats can mimic the galactopoietic effect of GH. While one study showed a small increase in milk synthesis (26), another study, using a slightly different experimental protocol, was without effect (27). It appears, therefore, that we cannot simply attribute the galactopoietic effect of GH treatment to a rise in plasma IGF-I.

Although IGF-II levels in plasma and milk are generally higher than IGF-I levels, the concentrations of IGF-II in plasma or milk were not affected by hGH treatment and none of the IGF-II measurements correlated with the milk volume response. It is therefore unlikely that IGF-II is causally involved in the mediation of GH-enhanced galactopoiesis. However, as IGF-II mRNA has been detected in mammary tissue (12), IGF-II may play a permissive role in lactation.

Our data show a significant increase in plasma IGFBP-3 levels with hGH treatment. IGFBP-3 levels started to rise very slowly at 24 h after the commencement of hGH treatment and continued to rise further during the course of the study. This increase in plasma IGFBP-3 commenced after the rise in plasma IGF-I, which showed a maximal increase within 3 days of hGH treatment. A similarly slow rise in plasma IGFBP-3 after hGH treatment has been observed in GH-deficient children (28). In light of the more rapid change of IGF-I after hGH injection, it has been proposed that the rise in plasma IGF-I may stimulate the increase in plasma IGFBP-3. And, indeed, hypophysectomized rats show an increase in plasma IGFBP-3 after bGH or IGF-I treatment (29). However, recently we have treated sheep with an intact somatotrophic axis with rhIGF-I for 8 weeks and observed no change in plasma IGFBP-3, while significant changes in plasma IGFBP-1, IGFBP-2 and metabolic parameters were observed (30). It thus appears that a rise in plasma IGF-I does not necessarily induce a rise in plasma IGFBP-3. The rise in plasma IGFBP-3 after hGH treatment therefore may be stimulated directly by hGH or, alternatively, it may depend on the state of the somatotrophic axis as to whether IGF-I can or cannot increase plasma concentrations of IGFBP-3. Species-specific differences cannot be excluded.

A comparison, in molar terms, of the increase in plasma IGF-I and plasma IGFBP-3 after hGH treatment reveals that IGF-I is increased by 38 nmol/l and IGFBP-3 by 47 nmol/l. This suggests that the increasing amounts of IGF-I in plasma after hGH treatment were most likely associated with IGFBP-3. The highly significant correlation between plasma concentrations of IGFBP-3 and IGF-I in this study supports this notion. As the 150-kd complex of plasma IGFBP-3 does not cross the capillary endothelium (4, 9), it has traditionally been viewed to serve as the endocrine storage depot for IGFs, increasing the half-life of IGF peptides in the circulation and limiting IGF access to the extravascular space (4, 9). Conversely, purified IGFBP-3 has been reported to enhance IGF-I action in vivo by means of cell-surface association, which decreases its affinity for IGF-I (31). In addition, there is recent evidence that binary IGFBP-3–IGF-I complexes might traverse the capillary endothelium, permitting delivery of IGF-I to target tissue (4, 32). The increase in plasma IGFBP-3 with hGH treatment, as observed in the present study, may thus facilitate the increased passage of IGF-I through the capillary system and could represent a mechanism for enhancing IGF-I delivery to mammary tissue. This concept is supported by data of the present study because the pattern of IGF-I increase in milk after hGH treatment follows the pattern of the increase in plasma IGFBP-3. In addition, the increase in plasma IGFBP-3 correlated with the increase in milk volume. However, the lack of a correlation between plasma IGFBP-3 levels and IGFBP-3 in milk suggests that active transport across mammary epithelium does not occur.
for this binding protein. Although IGFBP-2 is the second most abundant IGFBP in adult plasma, its concentration is about tenfold lower in comparison to IGFBP-3 and is reduced by hGH treatment. A negative relationship between plasma concentrations of GH and plasma IGFBP-2 have been observed in other studies (33) and it has been suggested that IGFBP-2 may mediate IGF transport and tissue distribution (34).

IGFBPs have been demonstrated previously in human (7), bovine (35) and rat milk (36) and are secreted by bovine mammary cells in culture (23). While a previous report (37) suggested that IGFBP-1 may be an important IGFBP in human milk, our study shows that the predominant IGFBP in milk is IGFBP-2, while IGFBP-1 and IGFBP-3 are present in much lower concentrations. A comparison, in molar terms, of total IGF concentrations (approximately 2.5 nmol/l) and IGFBP-2 concentrations (approximately 70 nmol/l) in milk shows a massive excess of free IGFBP-2 in milk. The tenfold higher concentrations of milk IGFBP-2 in comparison to plasma levels in our study suggest that IGFBP-2 is synthesized in mammary epithelium rather than transported from maternal circulation. This is in agreement with data in the rat (36), where IGFBP-2 is absent or very low in plasma but high concentrations of IGFBP-2 are found in milk. The notion that milk IGFBP-2 may arise from synthesis within the mammary gland is supported by observations of high levels of IGFBP-2 mRNA in normal rat mammary tissue (12) and the secretion of IGFBP-2 in human breast tumour cell lines (13). Our observation of high concentrations of IGFBP-2 in milk suggests that IGFBP-2 produced by mammary epithelial cells may play a role in determining regional levels and biological activity of IGF-I within the mammary gland.

In summary, our data show that hGH stimulates galactopoiesis in normally lactating women. Although our data support the hypothesis that IGF-I may be a principal mediator of the galactopoietic effect of GH, we cannot simply attribute the effect of GH solely to a systemic rise in IGF-1. The increase in plasma IGFBP-3 with hGH treatment suggests that IGFBP-3 could facilitate the delivery of IGF-I to the mammary gland. The high concentrations of “free” milk IGFBP-2 suggest that mammary epithelial IGFBP-2 may direct regional tissue distribution of IGF-I to the site of milk synthesis. The well-documented rise in cardiac output and mammary blood flow with GH treatment (1, 38) will supply the mammary gland with an increased amount of substrates to accommodate the demand required for enhanced milk synthesis.

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