Interaction between growth hormone and insulin in the regulation of lipoprotein metabolism in the rat

FREDRIK FRICK,1* DANIEL LINDÉN,1* CAROLINE AMÉÉN,1 STAFFAN EDÉN,1 AGNETA MODE,2 AND JAN OSCARSSON1

1Department of Physiology, Göteborg University, S-405 30 Göteborg; and 2Department of Medical Nutrition, Karolinska Institutet, Novum, S-141 86 Huddinge, Sweden

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IT IS WELL KNOWN THAT GROWTH HORMONE (GH) has marked effects on lipid and lipoprotein metabolism (1, 30). GH also increases secretion (27, 32) and plasma levels of insulin in humans and rats (31, 32, 37, 44). Moreover, GH increases DNA synthesis and proliferation of β-cells and insulin secretion in vitro (27), showing that GH enhances β-cell function independently of its insulin-antagonistic action (34, 35, 45). The increased serum insulin levels and the insulin-antagonistic effect of GH may be of importance for several effects of GH in vivo, but few studies have addressed this question (31, 36). Treatment of normal rats with the combination of insulin and GH results in an additive effect on body weight gain. However, GH treatment antagonizes the stimulatory effects of insulin on food intake and adipose tissue weight, indicating a complex interaction between GH and insulin (36).

The interaction of insulin and GH in the regulation of lipid and lipoprotein metabolism is of special interest, because similar effects of GH and chronic hyperinsulinemia have been observed (3, 4, 25, 42, 46, 47). GH treatment in vivo increases editing of apoB mRNA, production of apolipoprotein (apoB)-48, and very low-density lipoprotein (VLDL) secretion from isolated hepatocytes (42, 43) and perfused liver (10). Furthermore, triglyceride synthesis and secretion have been shown to increase in hepatocytes after GH treatment in vivo (7, 42) and in vitro (25). GH therapy of GH-deficient adults also increased VLDL-apoB secretion, an effect accompanied by increased serum insulin levels and insulin resistance, as indicated by increased hemoglobin A1C (3).

Insulin treatment in vivo has been shown to either increase or decrease the VLDL triglyceride secretion (18, 46). The different effects of insulin in vivo could be attributed to the nutritional and hormonal status of the subjects, including degree of insulin resistance (18, 23). In vitro, the duration of insulin incubation has been shown to be of importance. Short-term incubations of hepatocytes with insulin (up to 16 h) decrease VLDL secretion. However, exposure to insulin for longer periods of time (24–48 h) results in increased VLDL secretion (18, 23, 46, 49) and editing of apoB mRNA (47), i.e., effects similar to those of GH in vitro (25).

GH treatment was recently shown to increase hepatic gene expression of two key enzymes involved in fatty acid synthesis: fatty-acid synthase (FAS) and
stearoyl-CoA desaturase-1 (SCD-1) (12, 48). Insulin has been shown to induce FAS mRNA (38) and SCD-1 mRNA expression (28) in diabetic rodents. The effect of insulin on these enzymes has been attributed to increased gene expression of the transcription factor sterol regulatory element-binding protein (SREBP)-1 (13, 40) and has been reviewed (20, 39). SREBP-1 exists in two forms, SREBP-1a and SREBP-1c, which are transcribed from a single gene by the use of alternate promoters (20, 39). SREBP-1c predominates in most organs, including the liver. It has been demonstrated that hepatic SREBP-1 mRNA expression is increased in hyperinsulinemic insulin-resistant rodent models concomitant with increased hepatic lipogenesis (8, 41) and increased VLDL secretion (8).

In the present study, we used hypophysectomized (Hx) female rats that had decreased insulin secretion (26) as a nondiabeticogenic model to study the interplay of insulin and GH on lipid and lipoprotein metabolism. Moreover, we wanted to investigate whether the GH effects on lipogenic enzymes could be mediated by increased SREBP-1 gene expression.

MATERIALS AND METHODS

All chemicals used were from Sigma Chemicals (St. Louis, MO) if not stated otherwise.

Animals and Hormonal Treatment

Female Sprague-Dawley rats (Møllegaard Breeding Center, Ejby, Denmark) were hypophysectomized with a temporal approach at 50 days of age and maintained under standardized conditions of temperature (24–26°C), humidity (50–60%), and with lights on between 0500 and 1900. The rats had free access to standard laboratory chow (rat and mouse standard diet, B&K Universal, Sollentuna, Sweden) and water. Hormonal treatment started 7–10 days after hypophysectomy. All of the Hx rats were given cortisol phosphate (400 μg·kg⁻¹·day⁻¹; Solu-Cortef, Upjohn, Puurs, Belgium) and L-thyroxine (10 μg·kg⁻¹·day⁻¹; Nycomed, Oslo, Norway) diluted in saline as a daily subcutaneous injection (0800) (29, 43). Recombinant bovine GH was a generous gift from Amer-}

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Triglyceride and cholesterol concentrations in both serum and fast protein liquid chromatography (FPLC) fractions were determined by enzymatic colorimetric assays (Roche, Mannheim, Germany). The intra-assay coefficient of variation (CV) was 4% for the triglyceride assay and 3% for the cholesterol assay. Serum apoB and apoE concentrations were determined by electroimmunonassays as previously described (44). Serum glucose concentrations were measured by the glucose-6-phosphate dehydrogenase method (Merek, Darmstadt, Germany). Serum insulin concentration was determined by an RIA (human insulin RIA, Phe debas, Pharmacia Upjohn, Uppsala, Sweden) (31, 44).

Lipoprotein profiles were obtained by gel filtration using FPLC equipment (Pharmacia Upjohn) (14). Briefly, 250 μl of serum from 6 rats in each group were pooled to give a total volume of 1.5 ml, and the density was adjusted to 1.215 g/ml with KBr. After ultracentrifugation at 35,000 g for 24 h at 4°C, the removed supernatant, containing the total lipoprotein fraction, was adjusted with FPLC buffer (0.15 M NaCl, 0.01% EDTA, 0.02% sodium azide, pH 7.3) to 2 ml. After filtration through a 0.45-μm filter, the sample was loaded on a 25-ml Superose 6B column (Pharmacia Upjohn). The sample was eluted at a constant flow rate of 0.35 ml/min, and 0.5-ml fractions were collected. Triglyceride and cholesterol concentrations were determined with enzymatic colorimetric assays, as described above. The density (d) classes of lipoproteins obtained by sequential ultracentrifugation, VLDL (d < 1.006 g/ml), intermediate/low-density lipoprotein (d 1.006–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) are indicated in Fig. 1.

Direct Primer Extension Analysis

To analyze the extent of apoB mRNA editing, direct primer extension was performed as described previously (25, 43).

In Vivo Hepatic Triglyceride Secretion

Triglyceride secretion rate in vivo was measured by intravenous administration of Triton WR-1339 (24). The animals were fasted for 5 h (0700–1200) to avoid the influence of ongoing production of chylomicrons from the intestine. Thereafter, the rats were anesthetized and injected intravenously with Triton WR-1339 diluted in saline (200 mg/ml) via the tail vein (500 mg/kg body wt). Blood samples were taken before the injection (0 min) and 60, 120, and 180 min after Triton WR-1339 administration. Serum triglyceride levels were analyzed as described above, and hepatic triglyceride secretion rate was calculated from the slope of the curve and expressed as micromoles per hour per gram of body weight. The plasma volume was estimated to be 3.2% of the body weight, as described in normal rats (Jackson Laboratories, www.jax.org).

Quantification of mRNA

Total liver RNA was prepared using the Tri Reagent system.

FAS. Specific primers (5′-CTGAGACTCTTCTGGGCTACA-3′ and 5′-CGTCCCTGATCTCAAAGG-3′) amplified a 291-bp-long fragment of rat FAS cDNA (nt 250–540,
Normal female (N) and Hx rats not treated with GH or insulin served or insulin (INS) alone and in combination (GH + INS) for 7 days.

Table 1. Accession no. X13415, which was inserted into a pCR II-TOPO vector according to the manufacturer's protocol (TOPO TA Cloning kit, Invitrogen). The vector was linearized with EcoRI and a biotin-labeled antisense SREBP RNA probe was generated using Biotin-16-UTP and T7 RNA polymerase (Strip-EZ RNA, RNA probe synthesis kit, Ambion). The vector was linearized with EcoRI and a biotin-labeled antisense SREBP RNA probe was generated using Biotin-16-UTP and T7 RNA polymerase, as described above.

**SREBP-1.** A 257-bp fragment of rat SREBP-1 subcloned into PGEM 3Zf(+) (Promega, Madison, WI) was kindly provided by Dr. Joseph Goldstein, University of Texas Southwestern Medical Center, Dallas, TX. This probe allows measurement of both SREBP-1a and SREBP-1c (40). The vector was linearized with HindIII, and a biotin-labeled antisense SREBP RNA probe was generated using Biotin-16-UTP and T7 RNA polymerase, as described above.

**SCD-1.** A 200-bp fragment of rat SCD-1 (nt 4283–4482, accession no. J02585) was subcloned into PGEM 3Z (Promega, Madison, WI). We thank Cissi Gardno for making this construct. The vector was linearized with EcoRI and a biotin-labeled antisense SREBP RNA probe was generated using Biotin-16-UTP and T7 RNA polymerase, except for use of Sp6 RNA polymerase.

A biotin-labeled fragment of rat β-actin cDNA (Ambion) was used as an internal control in the gel ribonuclease protection assays (RPA). The levels of β-actin mRNA did not change as a result of the various hormonal treatments; β-actin was therefore regarded as an appropriate control. RPA was performed as described by the manufacturer (RPA III kit, Ambion). Protec-ted fragments were separated on denaturing 6% polyacrylamide Tris-boric acid-EDTA gels (Novex, San Diego, CA). For detection of biotin-labeled probes, protected fragments were transferred to Bright Star-Plus membranes (Ambion). After the transfer, the protected fragments were cross-linked by UV irradiation to the membrane, and detection was carried out using the Bright Star BioDetect Kit as described by the manufacturer (Ambion). The chemiluminescence was detected and quantified using the Fluor-S-Multimager. The amounts of the mRNA are expressed as a percentage, the ratio between the respective mRNA and β-actin mRNA.

**Statistical Analysis**

Values are expressed as means ± SE. Comparisons between groups were made by one-way analysis of variance (ANOVA) followed by Bonferroni’s test between individual groups. Values were transformed to logarithms when appropriate. A P value < 0.05 was considered significant.

**RESULTS**

**Weights and Serum Analyses**

Treatment with GH normalized the reduced weight gain of the Hx rats (Table 1). Insulin treatment increased the weight gain of Hx rats, but not to the level seen after GH treatment. Combined treatment with GH and insulin tended to increase the weight gain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight Gain, g/day</th>
<th>Retroperitoneal AT, % body wt</th>
<th>Ovarian AT, % body wt</th>
<th>S Glucose, mmol/l</th>
<th>S FFA, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.9 ± 0.3b</td>
<td>0.52 ± 0.07b</td>
<td>1.56 ± 0.12</td>
<td>8.29 ± 0.34a</td>
<td>0.49 ± 0.04a</td>
</tr>
<tr>
<td>Hx</td>
<td>0.3 ± 0.1a</td>
<td>0.56 ± 0.06b</td>
<td>1.00 ± 0.08b</td>
<td>7.05 ± 0.10a</td>
<td>0.41 ± 0.02b</td>
</tr>
<tr>
<td>Hx + GH</td>
<td>3.7 ± 0.3b</td>
<td>0.41 ± 0.03b</td>
<td>0.90 ± 0.06b</td>
<td>7.20 ± 0.10a</td>
<td>0.36 ± 0.02b</td>
</tr>
<tr>
<td>Hx + Insulin</td>
<td>1.1 ± 0.2</td>
<td>0.85 ± 0.06a</td>
<td>1.21 ± 0.09b</td>
<td>5.29 ± 0.51b</td>
<td>0.10 ± 0.3a</td>
</tr>
<tr>
<td>Hx + Insulin + GH</td>
<td>5.2 ± 0.3a</td>
<td>0.45 ± 0.05b</td>
<td>1.13 ± 0.07b</td>
<td>7.40 ± 0.20 a</td>
<td>0.38 ± 0.02b</td>
</tr>
</tbody>
</table>

Female rats were hypophysectomized (Hx) at 50 days of age. After 7–10 days of observation, rats were hormonally treated for 7 days. All Hx rats were given L-thyroxine (10 μg/kg · day−1) and cortisol phosphate (400 μg/kg · day−1) as a daily sc injection. Growth hormone (GH, 1.5 mg/kg · day−1), was given as a continuous infusion by means of osmotic mini pumps. Insulin was given as a daily sc injection at 1690, with dose gradually increased: days 1 to 4: 1.0 U/day, days 5 to 7: 2.0 U/day. Animals were killed between 0900 and 1100 without prior fasting. Serum(s) glucose levels were measured by a glucose-6-phosphate dehydrogenase assay. Serum free fatty acid (FFA) levels were determined by an enzymatic colorimetric assay. AT, adipose tissue. Values are means ± SE. Values with different superscripts are significantly different from each other (n = 6–7, P < 0.05, one-way ANOVA followed by Bonferroni’s test).
The ovarian adipose tissue weight was increased by insulin treatment, but this effect was not significantly affected by GH. The ovarian adipose tissue weight was in-creased by insulin treatment, but this effect was not significantly affected by GH or insulin treatment (Table 1). In this experiment, the rats were killed at 0900–1100. The serum level of insulin was 33.4 ± 10 mU/l in the group of Hx rats given insulin treatment compared with 8.0 ± 0.9 mU/l in Hx control rats when measured with a human insulin RIA. In Hx rats killed during the same diurnal period, serum insulin levels measured with a rat insulin RIA have been shown to be 13.7 ± 5 mU/l (15). Because the rats were killed 17–19 h after the last insulin injection, these results indicate that treatment with this slow-release form of insulin resulted in increased diurnal insulin levels. Serum glucose concentrations were not affected by Hx or GH treatment but decreased after insulin treatment (Table 1). Combined treatment with GH and insulin restored the serum levels of glucose, indicating an insulin-antagonistic effect of GH on glucose metabolism. Neither GH nor insulin had any effect on serum free fatty acid (FFA) levels compared with normal rats (Fig. 1A). GH slightly increased the cholesterol and triglyceride content of the LDL fraction, an effect of insulin that was not observed in the presence of GH (Fig. 1, A and B).

GH treatment normalized the increased serum apoB levels and the decreased serum apoE levels observed in the Hx rats (Table 2). Insulin treatment alone had no effect on serum levels of apoB or apoE, and insulin treatment did not influence the effects of GH treatment (Table 2).

### Hepatic Triglyceride Content and Secretion

To study the interaction between the effects of GH and insulin on hepatic triglyceride secretion in vivo, the rats were injected with Triton WR-1339 after 5 h of fasting (Fig. 2, A and B). The accumulation of triglycerides in serum after injection of Triton WR-1339 in the different groups of rats is illustrated in Fig. 2A. The hepatic triglyceride secretion rate was calculated and shown to be lower in Hx rats compared with normal rats. The hepatic triglyceride secretion rate increased ~70% by GH treatment (Fig. 2B). On the other hand, insulin treatment of Hx rats resulted in a trend toward lower hepatic triglyceride secretion. When GH and insulin were given together, the hepatic triglyceride secretion was markedly lower than when GH was given alone (Fig. 2B). Triglycerides and FFA concentrations in the serum samples taken before injection of Triton WR-1339 are shown in Fig. 2C. No significant effects of the various hormonal treatments on serum FFA levels were observed, in line with the results in the previous experiment. However, serum triglycerides were significantly lower when GH and insulin were given in combination (Fig. 2C). This result indicates that a short-term fast may influence the effect of combined GH and insulin treatment on serum triglycerides (see for example Table 2 and Fig. 2C).

To investigate whether the triglyceride content of the liver changed in parallel with the hepatic triglyceride secretion, hepatic triglyceride content was measured (Fig. 2D). Hepatic triglyceride content was influenced in a manner similar to triglyceride secretion after the hormonal treatments. GH treatment increased and insulin treatment decreased the hepatic triglyceride content. Moreover, when GH and insulin were given together, the hepatic triglyceride content was markedly lower than when GH was given alone (Fig. 2D).
ApoB mRNA Editing, SREBP-1, and Downstream Genes

ApoB mRNA editing in hepatocytes has been shown to increase by GH treatment in vivo (43) and in vitro (25). Moreover, editing of apoB mRNA has been shown to increase in the hyperinsulinemic Zucker rats (9) and by long-term insulin incubation of hepatocytes (25, 47). Therefore, apoB mRNA editing was measured (Fig. 3).

All mRNA levels were determined in the rats that were not fasted before they were killed, i.e., those described in Tables 1 and 2. The Hx rats had decreased apoB mRNA editing compared with normal rats. GH treatment increased the editing of apoB mRNA without (apoB-100 to apoB-48 mRNA from 1:0.6 to 1:1.3) and with combined insulin treatment (from 1:0.85 to 1:1.3). Insulin treatment also slightly increased the editing of apoB mRNA (from 1:0.6 to 1:0.85) (Fig. 3).

Both GH (12, 48) and insulin (28, 38) have been shown to increase FAS and SCD-1 gene expression in the liver. To address the question of an interaction between GH and insulin, we measured the expression of these gene products. Compared with normal female rats and in Hx female rats treated with GH or insulin alone or with GH and insulin in combination, FAS and SCD-1 mRNA levels were determined (Fig. 3).

The data were analyzed using one-way ANOVA followed by Bonferroni’s test. Values are means ± SE for 5–6 rats/group. Values with different superscripts are significantly different from each other (P < 0.05, one-way ANOVA).
rats, Hx rats expressed lower levels of FAS and SCD-1 mRNA (Fig. 4, A and B). GH treatment normalized both FAS and SCD-1 mRNA expression. Insulin treatment tended to increase FAS mRNA and significantly increased SCD-1 mRNA, but the effects of GH and insulin were not additive. Because increased FAS and SCD-1 gene expression has been shown to be mediated by increased expression of SREBP-1 (20, 39), we investigated the effect of GH on SREBP-1 expression. Compared with normal female rats, there was a trend toward a decreased expression of SREBP-1c mRNA in Hx rats. GH treatment resulted in an 85% increase in SREBP-1c mRNA expression (Fig. 4C). Insulin had no effect alone, but the effect of GH was blunted by concomitant insulin treatment (Fig. 4C). We also measured hepatic expression of SREBP-1a mRNA, but none of the hormonal treatments affected SREBP-1a gene expression (data not shown). Because GH may also affect SREBP-1 gene expression in adipose tissue, the effect of Hx and GH on adipose tissue expression of SREBP-1c mRNA, FAS mRNA, and SCD-1 mRNA was determined. There was no significant effect of Hx or GH treatment on SREBP-1c, FAS, or SCD-1 mRNA in adipose tissue (Table 3). As in the liver, there was no effect of Hx or GH treatment on SREBP-1a mRNA expression in adipose tissue (data not shown). Thus GH had no effect on SREBP-1c, FAS, or SCD-1 mRNA expression in adipose tissue, in contrast to the stimulatory effect of GH in the liver (Fig. 4C).

**DISCUSSION**

The present study was designed to investigate the interaction between GH and insulin in vivo on lipid and lipoprotein metabolism in a nondiabetogenic rat model. We found that insulin treatment did not affect most of the observed effects of GH on serum lipoproteins or apolipoprotein levels, indicating that these GH effects are not a result of changed insulin action. In contrast, the increased hepatic triglyceride secretion observed after GH treatment was blunted by concomitant insulin treatment, indicating that insulin and GH have antagonistic effects in terms of hepatic triglyceride secretion in vivo. Changed hepatic triglyceride content and expression of SREBP-1c mRNA paralleled the effect of GH on triglyceride secretion, indicating that the increased triglyceride secretion is at least partly mediated by increased triglyceride synthesis as a re-

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**Table 3. Effects of hypophysectomy and GH treatment on SREBP-1c, FAS, and SCD-1 mRNA levels in ovarian adipose tissue**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SREBP-1c/β-actin mRNA</th>
<th>FAS/β-actin mRNA</th>
<th>SCD-1/β-actin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100 ± 14.1</td>
<td>100 ± 5.6</td>
<td>100 ± 4.5</td>
</tr>
<tr>
<td>Hx</td>
<td>142 ± 17.7</td>
<td>128 ± 14.9</td>
<td>104 ± 4.3</td>
</tr>
<tr>
<td>Hx + GH</td>
<td>130 ± 14.0</td>
<td>113 ± 10.4</td>
<td>111 ± 8.0</td>
</tr>
</tbody>
</table>

Animals were treated as described in Table 1. mRNA levels of sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD-1) were measured with gel ribonuclease protection assays, as described in MATERIALS AND METHODS, and are expressed as a percentage of the respective mRNA to β-actin mRNA of normal rats. Values are means ± SE of 3–5 rats group. P values (one-way ANOVA) were 0.18 for SREBP-1c mRNA, 0.18 for FAS mRNA, and 0.42 for SCD-1 mRNA.
result of increased SREBP-1c expression. Compared with the effect of GH alone, concomitant GH and insulin treatment resulted in lower hepatic triglyceride content, lower triglyceride secretion, and lower gene expression of SREBP-1c. These findings suggest that the effect of GH on hepatic triglyceride secretion and SREBP-1c mRNA expression is dependent on decreased insulin action. Thus the increased expression of SREBP-1c and increased triglyceride secretion after GH treatment resemble the insulin-resistant state in, e.g., the obese JCR:LA-cp rats (8). In contrast to the effect of insulin treatment on hepatic content and secretion of triglycerides and SREBP-1c mRNA expression, insulin treatment of the Hx rats increased apoB mRNA editing and SCD-1 mRNA expression. GH had a smaller effect on apoB mRNA editing and SCD-1 mRNA when GH treatment was combined with insulin treatment. This finding indicates either that insulin antagonizes the effect of GH or that part of the effect of GH on apoB mRNA editing and SCD-1 mRNA expression is mediated by increased insulin secretion. In contrast to the findings in the liver, GH had no effect on SREBP-1, FAS, or SCD-1 mRNA in adipose tissue, showing a differential regulation of these genes by GH in the liver and adipose tissue. These results emphasize that GH has a liver-specific lipogenic effect.

The insulin treatment regimen used in this study has been shown to increase lipoprotein lipase activity in adipose tissue (31) and to increase incorporation of glucose into triglycerides in isolated adipose tissue segments (15). Thus the adipose tissue responded to this insulin treatment with an increased triglyceride accumulation, in contrast to what was observed in the liver. In line with this observation, infusion of 1.4 U/day of insulin for 7 days to intact rats has been shown to increase lipogenesis in adipose tissue but not in the liver (22). Together, these results emphasize that administration of insulin specifically enhances lipogenesis in adipose tissue.

Our observation of increased hepatic triglyceride secretion rate after GH treatment is in line with previous observations that GH increases triglyceride secretion ex vivo (10, 42) and in vitro (25). We extend these findings by showing that GH increases hepatic triglyceride secretion in vivo. Moreover, GH treatment in vivo increased the hepatic triglyceride content, which is in line with our previous finding that GH incubation of hepatocytes increased the triglyceride content of the cultured cells (25). Thus the effect of GH on hepatic triglyceride secretion in vivo is, at least partly, due to an increased triglyceride production.

Despite an increased triglyceride secretion from the liver after GH treatment, VLDL triglyceride levels were modestly affected. This finding may be explained by the increase in apoB mRNA editing. Increased apoB mRNA editing enhances the turnover of VLDL due to decreased proportion of apoB-100 containing VLDL (24, 25, 43). Moreover, lipoprotein lipase activity in skeletal muscle and heart is of major importance for the turnover of VLDL and serum VLDL levels (50), and these activities are increased by GH treatment (31).

We have observed that the combined GH and insulin treatment of Hx rats results in increased lipoprotein activity in both adipose tissue and muscle tissues (J. Oscarsson and S. Edén, unpublished results). This finding, together with the blunted hepatic triglyceride secretion, could be the reason for the low serum triglyceride levels after combined GH and insulin treatment in the fasted animals. Our finding that insulin treatment had small or no effects on serum lipid and lipoprotein levels is in line with previous studies in which intact rats were treated with a similar dose of insulin (22).

The increased gene expression of FAS and SCD-1 after GH treatment most likely contributed to the increased hepatic triglyceride content and secretion. Because the expression levels of FAS and SCD-1 mRNA were not different between the group of rats given GH alone and the rats given GH in combination with insulin, the inhibitory effect of insulin on hepatic triglyceride secretion could not be explained by changed gene expression of these enzymes. The inhibitory effect of insulin on GH-induced triglyceride secretion could be due to a decreased flux of FFA to the liver and/or inhibition of other enzyme activities that are upregulated by GH. Indeed, incubation with insulin has been shown to counteract the stimulatory effect of GH on phosphatidate phosphohydrolase activity in cultured rat hepatocytes (33). Thus insulin treatment may reduce the effect of GH on triglyceride secretion via a decreased supply of FFA for triglyceride synthesis and by inhibiting the effect of GH on phosphatidate phosphohydrolase activity.

Insulin treatment has been shown to increase both SCD-1 mRNA (28) and FAS mRNA expression (38) in diabetic animal models. The rather small effects of insulin in this study could be due to several factors. One obvious reason could be that the effect of insulin was studied in Hx rats that are not deficient in insulin (28, 38). Another reason could be increased levels of insulin-antagonistic hormones such as glucagon and catecholamines, as indicated by the lower serum glucose levels observed after insulin treatment. These hormones increase the hepatocyte content of cAMP, which has been shown to antagonize the effect of insulin on both SCD mRNA (28) and FAS mRNA expression (38). Because glucose is a powerful regulator of SREBP-1c mRNA expression (13, 39), the low glucose levels could have contributed to the low SREBP-1c, SCD-1, and FAS mRNA expression after insulin treatment. Furthermore, glucose is regulating FAS mRNA levels by increasing FAS mRNA stability (38).

The effect of GH on hepatic SCD-1 mRNA levels is in line with the observation that a continuous infusion of GH increased SCD-1 mRNA in Hx male rats (12). However, divergent results exist concerning the effect of GH on hepatic FAS mRNA expression. GH given as a continuous infusion, thus mimicking the female secretory pattern of GH (6), resulted in increased FAS mRNA levels as observed in this study and in intact male rats (48). On the contrary, GH given as daily injections to ovariectomized female rats, mimicking
the male secretory pattern of GH, decreased FAS mRNA expression (5). In this context, it is interesting to note that only GH given as a continuous infusion could fully restore triglyceride synthesis and VLDL secretion in Hx rats to those of normal rats (42).

We observed no effect of GH on gene expression of SREBP-1, FAS, or SCD-1 mRNA in adipose tissue. This finding is in line with the observation that GH does not influence SREBP-1 expression in cultured rat preadipocytes (19). To the best of our knowledge, the effect of GH on adipose tissue SCD-1 mRNA has not previously been studied. However, GH has been shown to decrease FAS mRNA in cultured rat preadipocytes (19), cultured 3T3-F442A cells, and pig adipose tissue (11). The reason for these discrepant data is not clear but may depend on differences between species or experimental conditions.

In summary, GH treatment increased triglyceride content and secretion and increased hepatic gene expression of SREBP-1c, FAS, and SCD-1 mRNA. In contrast, Hx and GH treatment had no effect on the expression of SREBP-1c or its downstream genes in adipose tissue. The most prominent effect of insulin was to mitigate the effect of GH on hepatic lipogenesis, as indicated by the finding that insulin inhibited the effect of GH on hepatic triglyceride secretion, triglyceride content, and SREBP-1c mRNA expression. Thus the effects of insulin in GH-treated Hx rats resemble the previously observed inhibitory action of insulin on hepatic triglyceride secretion (2, 18, 46, 49). Furthermore, it is noteworthy that the effect of insulin on SREBP-1c mRNA expression differs between the non-diabeticogenic rat model used in this study and diabeticogenic rat models (40).

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REFERENCES


