Effects of gender and GH secretory pattern on sterol regulatory element-binding protein-1c and its target genes in rat liver

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GROWTH HORMONE (GH) plays an important role in the regulation of lipid metabolism. GH has lipolytic and antilipogenic effects in adipose tissue (6) that contribute to the decreased body fat mass observed after GH treatment (2, 42). Elevation of plasma GH levels also results in increased plasma insulin levels and insulin resistance (6). The increased insulin secretion can be explained by a direct effect of GH on the pancreatic β-cells (33, 40), whereas the insulin resistance may be explained by increased fatty acid oxidation in some (34), but not all, situations (18, 37).

GROWTH HORMONE (GH) secretion from the pituitary is sexually differentiated in all mammals, but the difference is particularly pronounced in rodents (8, 17, 21). In female rats, the GH secretion is frequent and nearly continuous with high basal levels, whereas GH is secreted as regular, high-amplitude pulses with low or undetectable levels between peaks in males (8, 46). Several hepatic functions that are sex differentiated are regulated by the sexually dimorphic secretory pattern of GH in rodents (1, 32, 45, 53). A continuous infusion of GH for 7 days to hypophysectomized (Hx) rats, thus mimicking the female GH secretory pattern, increased hepatic triglyceride synthesis (9) and VLDL secretion from isolated perfused livers (11). We (45) extended these findings by showing that continuous administration of GH, but not two daily injections of GH, resulted in increased triglyceride biosynthesis and VLDL assembly in isolated rat hepatocytes. These findings could explain the observation that female rats have higher hepatic triglyceride biosynthesis and secretion than male rats (39, 52).

The mechanism behind the specific effect of the female characteristic continuous GH exposure on hepatic triglyceride synthesis is not known. Our laboratory (14) has shown that a continuous infusion of GH to Hx rats increases hepatic triglyceride secretion and content as well as mRNA expression of sterol regulatory element-binding protein-1c (SREBP-1c) and the lipogenic enzymes fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD-1). SREBP-1c is known to activate the transcription of both FAS and SCD-1, as well as acetyl-CoA carboxylase-1 (ACC1) and the rate-limiting enzyme in glycerolipid formation, glycerol-3-phosphate acyltransferase (GPAT) (20, 43). The effect of insulin on these lipogenic genes in the liver is a result of increased expression of SREBP-1c mRNA (20, 43). SREBP-1c gene expression is also paradoxically upregulated in insulin-resistant hyperinsulinemic models (10, 20, 44). Recently, the effect of insulin on expression of SREBP-1c and its downstream genes was shown to be dependent on the presence of liver X receptors (LXRα) (48), members of the nuclear hormone receptor family that are activated by oxysterols (29). LXR activation results in diverse effects on cholesterol metabolism as well as increased hepatic lipogenesis that is largely due to increased expression of SREBP-1c (20, 29). Moreover, insulin treatment increases hepatic LXRα expression (48). It is therefore conceivable that the effect of GH on the hepatic expression of SREBP-1c and its downstream target genes involved in fatty acid synthesis (13, 14, 49, 50)

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also would be mediated through changes in LXRα expression levels (20, 43).

The aims of this study were to investigate whether there is a sex difference in mRNA expression levels of SREBP-1c, ACC1, FAS, SCD-1, and GPAT and to study whether these genes are regulated by the sex-differentiated GH secretory pattern. Moreover, we studied whether GH regulates LXRα expression and whether feminization of the GH secretory pattern results in decreased insulin sensitivity.

MATERIALS AND METHODS

Animals and hormonal treatment. Normal and hypophysectomized (Hx) Sprague-Dawley rats were purchased from Mollegaard Breeding Center (Ejby, Denmark). They were maintained under standardized conditions of temperature (24–26°C) and humidity (50–60%), with light from 0500 to 1900. The rats had free access to water and standard laboratory chow containing (wt/wt) 4% fat, 58% carbohydrates, 16.5% protein, and 6% ash, with a total energy content of 12.55 kJ/g (Lactamin R-34; Lactamin, Stockholm, Sweden).

Female rats were hypophysectomized with a temporal approach at 21–22 days of age. Normal rats were age matched. A weight gain of 12.55 kJ/g/day among Hx rats during a 7- to 10-day observation period (E1040 SREBP-1C, GH, AND LIVER

A bolus injection of insulin (32.7 nU Actrapid; Novo Nordisk, Bagsværd, Denmark), insulin was continuously infused at a rate of 5 μM·min⁻¹·kg⁻¹. A 10% glucose solution in physiological saline was administered to maintain plasma glucose concentration at 6 mM. Glucose was measured in 10-μl blood samples at regular intervals (every 5 min during the first 40 min and then every 10 min). Blood samples were also taken at 80 and 90 min for determination of insulin concentration and [3H]. The blood samples (100 μl) taken at 0, 80, and 90 min were deproteinized, evaporated, and then resuspended in deionized water for determination of radioactivity and glucose levels. The total blood volume taken from each animal was 1.5 ml. The mean glucose infusion rate was calculated on the basis of values from the last 60 min of the clamp. Glucose turnover rate was calculated from the radioactivity of the [3H]glucose infusion (dpm/μl) times the glucose infusion rate (μl/min) divided by the specific radioactivity of glucose (dpm/mg) and body weight (kg). Hepatic glucose production was calculated by subtracting the glucose infusion rate from the glucose turnover rate.

Serum analyses. Blood was collected in heparinized microtubes and immediately centrifuged for plasma preparation. Plasma insulin was analyzed with a rat insulin ELISA kit (Mercodia, Uppsala, Sweden). Glucose concentrations were measured in whole blood with the B-glucose Analyzer Hemocue (HemoCue, Dronfield, Derbyshire, UK).

Hepatocyte cultures and hormonal treatment. Hepatocytes were obtained by nonrecirculating collagenase perfusion through the portal vein of female Sprague-Dawley rats weighing 200–300 g as described (3). In brief, the cells were seeded at ~120,000 cells/cm² in 100-mm dishes (Falcon, Plymouth, UK) coated with laminin-rich Matrigel (BD Biosciences, Bedford, MA). The cells were plated for 16–18 h in Williams’ E medium with Glutamax (Invitrogen, Carlsbad, CA) supplemented with penicillin (50,000 IU/ml, Invitrogen), streptomycin (50 mg/l, Invitrogen), 0.28 mM sodium ascorbate (Sigma Chemical, St. Louis, MO). 1 μM sodium selenite (Sigma), 3 g glucose/l (final concentration 28 mM, Sigma), and 16 mM insulin (Actrapid, Novo Nordisk) (3). The cells were then treated for 3 days with bovine GH (100 ng/ml) in a medium supplemented as described above except for the exclusion of insulin and the addition of 1 nM dexamethasone (Sigma). In other experiments, the culture medium was also supplemented with 50 nM triiodothyronine (Sigma), 3 nM insulin (Actrapid), or 500 mM oleic acid (Sigma) in combination with 0.75% essentially fatty acid-free bovine serum albumin (Sigma). We also investigated the effect of GH in a medium without extra supplementation of glucose (final concentration 5 mM).

Quantification of RNA. Total RNA from frozen liver and cultured hepatocytes was isolated with Tri-Reagent according to the manufacturer’s protocol (Sigma). The RNA concentration was determined spectrophotometrically at 260 nm.

Ribonuclease protection assay. The generation of bovine-labeled antisense probes for SREBP-1, SCD-1, and β-actin is described in Ref. 14. A bovine-labeled antisense probe for ACC1 was generated by amplifying a 280-bp-long fragment of ACC1 (accession no. J03808) with the specific primers 5′-GTGGTGATATAAGGCTC-3′ and 5′-GGATACCTTCGACGAC-3′ and inserting it into a pCR II-TOPO vector (TOPO TA Cloning kit, Invitrogen). HindIII was used to linearize the vector, and a bovine-labeled antisense ACC1 RNA probe was generated using bovine-16-UTP (Enzo; Roche, Basel, Switzerland) and T7 RNA polymerase (Strip-EZ RNA, RNA probe synthesis kit; Ambion, Austin, TX). Rat β-actin was used as an internal control in the ribonuclease protection assay (RPA). The level of β-actin mRNA was not regulated by sex or the various hormonal treatments used in this study. The RPA probes were hybridized to the sample RNA by use of an RPA III kit (Ambion), and the protected fragments were separated and detected as described before (1, 14). The amounts of the transcripts are expressed as the ratio between the gene of interest and the internal control (β-actin).

cDNA synthesis and real-time PCR. Recombinant DNase I (Ambion) was used to remove DNA from the RNA preparations. First-
strand cDNA was synthesized from 2 μg of total RNA by means of random hexamers and the SuperScript protocol (Life Technologies, Carlsbad, CA). Specific primer/probe sets were designed with Primer Express software (Applied Biosystems, Foster City, CA) and gene sequences from GenBank. To avoid amplification of genomic DNA, the primers were positioned to span exon junctions. Primers and fluorogenic probes were synthesized by Applied Biosystems, and the sequences of the primers and the probes are as follows. Mitochondrial GPAT (acc. no. U36771): forward (F) 5’-CCACCCACATTGTCGCCT-3’, reverse (R) 5’-GAGAGTGATCCCCTGCCT-3’, probe (P) 5’-CCCTGCCTCTTACAGACA-3’; LXa: F 5’-GCTCTGCTCATGGCCATCG-3’, R 5’-TGTTGCAGTCTCTCTACTTGGA-3’, P 5’-TCTGCAGACCGGCCCAACGTG-3’; acidic ribosomal phosphoprotein P0 (36B4; acc. no. X15096): F 5’-TTCCACCTGGTGAAGAGGT-3’, R 5’-CGGAGCGCAGAAATGC-3’, P 5’-AGGCTTCTGGCCGCATCCATC-3’.

Table 1. Final body weight and body weight gain in N and Hx female rats administered GHc or 2×GH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Body Weight, g</th>
<th>Body Weight Gain, g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>241.6±2.9*</td>
<td>4.47±0.18*</td>
</tr>
<tr>
<td>Hx</td>
<td>127.0±1.5</td>
<td>0.32±0.08</td>
</tr>
<tr>
<td>Hx + GHc</td>
<td>149.8±1.1*</td>
<td>1.87±0.06*</td>
</tr>
<tr>
<td>Hx + 2×GH 2 h</td>
<td>153.9±3.1*</td>
<td>2.08±0.11*</td>
</tr>
<tr>
<td>Hx + 2×GH 6 h</td>
<td>151.4±1.9*</td>
<td>2.07±0.08*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats per group. N, normal; Hx, hypophysectomized; GHc, growth hormone (GH) administered as a continuous infusion; 2×GH, GH administered as 2 daily injections; Rats were Hx at 42–43 days of age and given GHc or 2×GH for 7 days. Rats given GH injections were killed either 2 or 6 h after the last GH injection. *P < 0.05 vs. Hx, 1-way ANOVA followed by Tukey’s test.

Fig. 1. Hepatic mRNA expression of sterol regulatory element-binding protein-1c (SREBP-1c; A), acetyl-CoA carboxylase-1 (ACC1; B), fatty acid synthase (FAS; C), stearoyl-CoA desaturase-1 (SCD-1; D), and glycerol-3-phosphate acyltransferase (GPAT; E) in intact female and male rats. Hepatic mRNA levels were determined with ribonuclease protection assay (RPA; A–D) and real-time PCR (E). Values are means ± SE of 5 (A and E), 9–10 (B), and 13–14 (C and D) observations (*P < 0.05, t-test).
Real-time PCR was performed in an ABI Prism 7700HT sequence detection System (Perkin-Elmer Applied Biosystems) according to the manufacturer’s instructions. The reactions were analyzed in triplicate, and the correct sizes of the amplicons were verified by gel electrophoresis. The mRNA expression was normalized to an endogenous control, 36B4. The mRNA level of 36B4 was invariable in this study. The relative expression levels were calculated according to the formula

$$\frac{2^{-\Delta\Delta CT}}{H9004}$$

where \(\Delta CT\) is the difference in critical threshold (CT) values between the target and the 36B4 internal control.

Western blot. Total protein extracts from frozen livers were prepared as described (1). Protein concentrations were determined with the RC DC Protein Assay Kit II (Bio-Rad, Hercules, CA). Western blotting was performed using an enhanced chemiluminescence (ECL) protocol (Amersham Pharmacia Biotech). Fifteen micrograms of protein were separated on a 4–20% gradient polyacrylamide Tris-glycine gel (Novex, San Diego, CA). After electrophoresis, the proteins were transferred to a Hybond-P polyvinylidene difluoride transfer membrane (Amersham Pharmacia Biotech) in transfer buffer (25 mM Tris, pH 7.6, with 192 mM glycine and 25% methanol) for 2–2.5 h at 400 mA (Transblot cell, Bio-Rad). Equal loading was confirmed by staining the membrane with 0.2% Ponceau S (Serva, Heidelberg, Germany). The molecular mass standard Precision Plus Protein Dual Color Standards (Bio-Rad) was used. The membrane was blocked for 1 h at room temperature or overnight at 4°C in 50 mM Tris-buffered saline, pH 7.6, containing 0.1% Tween 20 (TBS-T) and 5% nonfat milk and then incubated for 1 h with polyclonal FAS (cat. no. 610962; BD Biosciences Pharmingen, San Diego, CA) diluted 1:500 or SCD-1 antibodies [kindly provided by Dr. Juris Ozols (38)] diluted 1:2,000 in TBS-T and 5% nonfat milk. The membrane was incubated for 1 h with peroxidase-labeled anti-mouse IgG (Amersham Life Science) diluted 1:10,000 in TBS-T and 5% nonfat milk. The membranes were washed three times in TBS-T and incubated with peroxidase-conjugated anti-mouse IgG at a dilution of 1:2,000. The protein bands were visualized using the ECL plus detection system (Amersham Life Science) and exposed to Kodak X-Omat film.
described in MATERIALS AND METHODS. There were 4 observations in each group. Because the expression of SREBP-1c mRNA expression (Fig. 1). Protein levels were determined with Western blot, as described in MATERIALS AND METHODS. There were 4 observations in each group. (*P < 0.05 vs. Hx, 1-way ANOVA followed by Tukey’s test).

1:7,500 or peroxidase-labeled anti-rabbit IgG (Amersham) diluted 1:5,000. Detection and development were performed using the ECL detection system. The chemiluminescence was measured using a Fluor-S-Multimager (Bio-Rad), and the band intensity was measured with ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The protein expression was normalized to an endogenous control, heat shock protein (HSP90α/β (H-114, sc-7947; Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:1,000. Peroxidase-labeled anti-rabbit IgG (Amersham) diluted 1:10,000 was used as the secondary antibody. The protein level of HSP90αβ was variable in this study.

**Statistical analysis.** Values are expressed as means ± SE. Comparisons between groups were made by t-test, one-way analysis of variance (ANOVA) followed by Tukey’s test, or two-way ANOVA using treatment and experiment as factors. Values were transformed to logarithms when appropriate. P < 0.05 was considered significant.

**RESULTS**

**Hepatic mRNA expression of SREBP-1c and its target genes in females and males.** Because the expression of SREBP-1c and its downstream target genes are important regulators of fatty acid and triglyceride synthesis, we investigated whether female rats have higher expression levels of these gene products than male rats. The SREBP-1c, FAS, and GPAT mRNA levels were higher in the liver of females compared with males, whereas there was no sex difference in ACC1 or SCD-1 mRNA expression (Fig. 1).

**Regulation of SREBP-1c and its target genes by continuous and intermittent GH plasma patterns.** To investigate the regulatory role of the GH secretory pattern for the expression of SREBP-1c and its downstream target genes, we used Hx female rats that were administered GH either as a continuous infusion (GHc) or as two daily injections (2 × GH) to mimic the female and the male GH secretory pattern, respectively. Final body weight and body weight gain were significantly increased in Hx animals by both modes of GH administration, albeit not to the level observed in intact female rats (Table 1). Hx decreased the mRNA levels of SREBP-1c, FAS, SCD-1, and GPAT mRNA but did not significantly change ACC1 mRNA expression (Fig. 2). Treatment of Hx rats with GHc increased the mRNA expression of SREBP-1c and all of its downstream lipogenic genes compared with Hx control rats (Fig. 2). 2 × GH, however, did not affect any of the genes except for SCD-1 mRNA expression, which was increased at both time points after the last GH injection (Fig. 2D). Thus the continuous female-like GH treatment increased the mRNA levels of SREBP-1c and all of the measured lipogenic genes (ACC1, FAS, SCD-1, and GPAT), whereas the male-like GH administration enhanced only SCD-1 mRNA expression. To investi-
SCD-1 mRNA expression in hepatocytes not mediated via changed SREBP-1c expression and that the effect of GH on SREBP-1c, FAS, and GPAT mRNA observed in vivo is indirectly mediated by another factor(s) that is influenced by GH treatment.

Effect of continuous GH infusion to intact male rats on lipogenic genes and insulin sensitivity. Because the expression of SREBP-1c mRNA and downstream lipogenic enzymes in the liver is upregulated in insulin-resistant animal models, we hypothesized that the female continuous pattern of GH secretion would result in lower insulin sensitivity than the intermittent GH secretory pattern of male rats. The degree of insulin sensitivity was measured with the euglycemic hyperinsulinemic clamp technique in 55-day-old male rats given GH (0.5 mg·kg$^{-1}$·day$^{-1}$) as a continuous infusion for 7 days. This dose is about one-third of the endogenous diurnal GH secretion (4, 24) and will increase the basal levels of GH between the endogenous GH peaks (4, 7). The treatment had no effect on body growth (Table 2) or hepatic IGF-I mRNA levels (data not shown). These results indicate that the total GH exposure was minimally affected and that the effects of this treatment can be regarded as effects of feminization of the GH secretory pattern. The GH infusion resulted in 1.8-fold higher nonfasting basal plasma insulin levels and a 32% decrease in glucose infusion rate (Table 2). The hepatic glucose output or glucose turnover was not significantly influenced by the GH infusion (Table 2). The achieved plasma levels of insulin during the clamp in the two treatment groups were similar (control, 91.0 ± 4.9 mU/l; GHc, 94.6 ± 6.4 mU/l).

To investigate the effect of changed GH secretory pattern on SREBP-1c and its downstream target genes in this model, a low dose of GH (0.5 mg·kg$^{-1}$·day$^{-1}$) was given as a contin-
GH given as a continuous infusion to male rats did not increase 18% (n = 11005) mRNA expression of FAS (male controls 100 mRNA levels (data not shown). However, the GH treatment male GHc, 7.4 were administered a low dose of GH (0.5 mg·kg⁻¹·day⁻¹) for 7 days. Basal insulin levels were determined in the nonfasting state before the insulin clamp was initiated. Insulin sensitivity was determined with the euglycemic insulinemic clamp technique as described in MATERIALS AND METHODS. Insulin was continuously infused at a rate of 5 µU·kg⁻¹·min⁻¹, and the plasma glucose level was maintained at 6 mM. *P < 0.05, t-test.

The different response of SREBP-1c, FAS, and GPAT mRNA to GH in vivo and in vitro indicated that the GH effect on these genes in vivo is indirect via a change in metabolism that does not occur in vitro. These results clearly emphasize the restricted value of in vitro experiments alone in terms of understanding gene regulation. One plausible indirect effect of GH that could upregulate these genes is increased insulin resistance, because insulin-resistant states are associated with increased expression of these genes (10, 44). The insulin resistance and hyperinsulinemia that occurred in response to continuous GH exposure could therefore be the underlying cause of the effects of the female GH secretion pattern on FAS and GPAT gene expression in vivo. This hypothesis is concordant with our previous finding that insulin treatment of Hx rats tended to increase FAS mRNA and that continuous GH infusion and insulin treatment had no additive effects on FAS mRNA levels (14). Others have also demonstrated that continuous GH infusion (0.5 mg·kg⁻¹·day⁻¹) to intact male rats results in hyperinsulinemia and a 31% decrease in glucose infusion rate (18). These authors also found that continuous infusion of GH results in decreased lipid oxidation and, in contrast to our study, in increased hepatic glucose output. The reason for this discrepancy is unclear but may be due to different experimental conditions, such as different strains of rats or differences in insulin infusion rates. Preliminary data from the same group also showed that whole body and liver insulin resistance occurred only upon continuous GH admin-

### DISCUSSION

It has previously been shown that female rats have higher triglyceride synthesis and secretion than male rats (39, 52). Moreover, triglyceride synthesis, triglyceride secretion, and VLDL assembly are specifically upregulated by the female pattern of GH exposure (45). These findings prompted us to study the effects of gender and the sexually dimorphic secretory pattern of GH on regulation of hepatic SREBP-1c and its downstream target genes (summarized in Table 3). The finding that female rats had higher expression of FAS and GPAT mRNA compared with male rats and that these genes were specifically increased in Hx rats by a female characteristic exogenous GH administration could, hence, explain the effect of the female pattern of GH secretion on hepatic triglyceride synthesis and secretion (9, 11, 14, 45). These findings might also be relevant in humans, as hepatic de novo palmitate synthesis has been shown to be higher in women than in men (12). Furthermore, the sex-differentiated GH secretion in humans (17, 21) is probably involved in the regulation of the plasma levels of lipoprotein (a) by influencing hepatic apo(a) mRNA levels (36, 47) and other human hepatic functions such as CYP3A4 activity (22). Thus these data indicate that there is a sex difference in hepatic lipogenesis and that hepatic functions are influenced by the sex-differentiated GH secretory pattern also in humans.

In line with increased FAS and GPAT mRNA levels, SREBP-1c mRNA, known to activate transcription of the FAS and GPAT genes (20, 43), was increased in Hx rats upon continuous GH infusion. However, although feminization of the GH plasma pattern by continuous infusion of GH to intact male rats also increased FAS and GPAT mRNA, the SREBP-1c mRNA level remained unchanged. This clearly shows that the FAS and GPAT genes can be upregulated by continuous GH without a concomitant increase in SREBP-1c mRNA. Even though the increase of FAS and GPAT mRNA expression by continuous GH infusion was evident in vivo, we did not see an upregulation of these genes by GH in vitro. In fact, FAS and GPAT mRNA levels were significantly decreased by GH incubation of primary hepatocytes, whereas SREBP-1c mRNA was unchanged. LXRα mRNA levels were also decreased in hepatocytes after GH exposure. Because the FAS gene contains LXRE response elements (LXRE) (25), it could be speculated that the decreased FAS mRNA expression would be mediated by reduced LXRE expression in vitro. A similar mechanism may also be involved in the downregulation of GPAT mRNA, but to our knowledge it is not known whether the GPAT gene contains any functional LXRE.

The different response of SREBP-1c, FAS, and GPAT mRNA to GH in vivo and in vitro indicated that the GH effect on these genes in vivo is indirect via a change in metabolism that does not occur in vitro. These results clearly emphasize the restricted value of in vitro experiments alone in terms of understanding gene regulation. One plausible indirect effect of GH that could upregulate these genes is increased insulin resistance, because insulin-resistant states are associated with increased expression of these genes (10, 44). The insulin resistance and hyperinsulinemia that occurred in response to continuous GH exposure could therefore be the underlying cause of the effects of the female GH secretion pattern on FAS and GPAT gene expression in vivo. This hypothesis is concordant with our previous finding that insulin treatment of Hx rats tended to increase FAS mRNA and that continuous GH infusion and insulin treatment had no additive effects on FAS mRNA levels (14). Others have also demonstrated that continuous GH infusion (0.5 mg·kg⁻¹·day⁻¹) to intact male rats results in hyperinsulinemia and a 31% decrease in glucose infusion rate (18). These authors also found that continuous infusion of GH results in decreased lipid oxidation and, in contrast to our study, in increased hepatic glucose output. The reason for this discrepancy is unclear but may be due to different experimental conditions, such as different strains of rats or differences in insulin infusion rates. Preliminary data from the same group also showed that whole body and liver insulin resistance occurred only upon continuous GH admin-

### Table 2. Effect of a low dose of GHc on insulin sensitivity

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>GHc (0.5 mg·kg⁻¹·day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>356±7.8</td>
<td>355±11.0</td>
</tr>
<tr>
<td>Body weight gain, g/day</td>
<td>8.2±0.5</td>
<td>8.5±0.9</td>
</tr>
<tr>
<td>Basal plasma insulin, pmol/l</td>
<td>662±86</td>
<td>1187±140*</td>
</tr>
<tr>
<td>Glucose infusion rate, mg·kg⁻¹·min⁻¹</td>
<td>11.1±1.2</td>
<td>7.5±0.8*</td>
</tr>
<tr>
<td>Glucose turnover, mg·kg⁻¹·min⁻¹</td>
<td>31.6±3.0</td>
<td>34.6±2.4</td>
</tr>
<tr>
<td>Hepatic glucose output, mg·kg⁻¹·min⁻¹</td>
<td>23.2±3.1</td>
<td>26.2±4.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10 rats/group. Intact 55-day-old male rats were administered a low dose of GH (0.5 mg·kg⁻¹·day⁻¹) for 7 days. Basal insulin levels were determined in the nonfasting state before the insulin clamp was initiated. Insulin sensitivity was determined with the euglycemic insulinemic clamp technique as described in MATERIALS AND METHODS. Insulin was continuously infused at a rate of 5 µU·kg⁻¹·min⁻¹, and the plasma glucose level was maintained at 6 mM. *P < 0.05, t-test.

### Table 3. Summarized effects of GH treatment in vivo and in vitro on hepatic gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Females vs. Hx</th>
<th>Hx + GHc</th>
<th>Hx + 2×GHc</th>
<th>GHc to Intact Males</th>
<th>GHc in Vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>SREBP-1c</td>
<td>↑</td>
<td>↑</td>
<td>∼</td>
<td>ND</td>
<td>↑</td>
</tr>
<tr>
<td>ACC1</td>
<td>∼</td>
<td>*</td>
<td>*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FAS</td>
<td>∼</td>
<td>*</td>
<td>*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SCD-1</td>
<td>∼</td>
<td>*</td>
<td>*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GPAT</td>
<td>∼</td>
<td>*</td>
<td>*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LXRα</td>
<td>∼</td>
<td>*</td>
<td>*</td>
<td>ND</td>
<td>ND</td>
</tr>
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</table>

SREBP-1c, sterol regulatory element-binding protein-1c; ACC1, acetyl-CoA carboxylase-1; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase-1; GPAT, glycerol-3-phosphate acyltransferase; LXRα, liver X receptor-α; ND, not determined. *mRNA and protein data.
istration and not after pulsatile GH administration to intact male rats (26). Together, these results strengthen the conclusion that feminization of the GH plasma pattern increases insulin resistance. However, we cannot conclude from the present results that changed insulin sensitivity is the cause of the changed expression of FAS and GPAT.

The GH pattern-dependent regulation demonstrated for FAS and GPAT mRNA did not apply to the regulation of ACC1 and SCD-1 mRNA. Moreover, the expression of these genes was not sex differentiated. In contrast to our finding in rats, SCD-1 mRNA expression is higher in female mice than in male mice (27). The reason for the difference between the species is not clear, but one possible explanation is the impact of the nuclear receptor peroxisome proliferator-activated receptor-α (PPARα) (30). In rats, hepatic PPARα expression is markedly higher in males than in females (23), whereas in mice the PPARα expression is similar in the sexes (54). Because PPARα upregulates SCD-1 mRNA levels (30), the higher expression of PPARα in male rats might counterbalance the effect of female sex on SCD-1 mRNA levels. We (14) and others (13) have previously reported increased hepatic expression of SCD-1 after continuous infusion of GH to Hx rats. In this study, we extend those findings by showing that SCD-1 mRNA, as well as SCD-1 protein expression, is upregulated by both female and male modes of GH administration to Hx rats. Moreover, SCD-1 mRNA levels were unchanged when the GH plasma pattern in intact males was feminized by administration of a continuous GH infusion. This finding is in line with a previous study using the same model (16). Thus these results show that the female GH secretory pattern does not specifically regulate SCD-1 mRNA expression.

In contrast to the other lipogenic genes, ACC1 mRNA expression was not reduced in Hx rats. Furthermore, the lack of effect of continuous GH infusion in intact males on ACC1 mRNA levels indicates yet a different regulation of this gene. It has been shown that 2-yr-old male rats have increased secretion of GH between GH pulses, resulting in increased hepatic expression of female-specific cytochrome P450 genes (7). Rats of the same age have increased FAS mRNA expression, whereas ACC mRNA levels are unchanged (50). It is therefore concluded that ACC1 is differently regulated from FAS. It is possible that other hormones present only in intact male rats suppress the action of continuous GH exposure on ACC1 and SREBP-1c mRNA, but not on FAS and GPAT mRNA expression.

We have previously observed that GH incubation of hepatocytes using the same culture conditions as used in this study results in a 50% increase in triglyceride synthesis (28). Taken together, our results indicate that increased expression of SREBP-1c, FAS, or GPAT mRNA is not necessary for an effect of GH on triglyceride biosynthesis in vitro. An explanation for this finding could be that GH has other effects on triglyceride synthesis that occur in vitro, e.g., increased phosphatidate phosphohydrolase activity (41), or that increased expression of SCD-1 is sufficient for increased hepatic triglyceride synthesis. The importance of SCD-1 for triglyceride synthesis is supported by the finding that SCD-1 knockout mice have decreased triglyceride levels (31) and that increased SCD-1 expression is responsible for the increased hepatic lipogenesis in ob/ob mice (5). However, increased expression of SCD-1 cannot explain the specific effect of the female secretory pattern of GH in vivo on hepatic triglyceride synthesis (45), because both modes of GH administration increased SCD-1 mRNA and protein. Thus different sets of genes could be responsible for the effect of GH on triglyceride synthesis in vivo and in vitro, or, alternatively, as-yet-unidentified mechanisms are responsible for the common effects in vivo and in vitro on triglyceride synthesis.

In summary, we have shown that the sexually dimorphic GH secretion in rat regulates hepatic FAS and GPAT mRNA levels. Moreover, we found that mRNA and protein levels of FAS and SCD-1 were regulated in parallel, showing that the GH regulation of these genes is primarily at the mRNA level. We found no evidence for the assumption that GH regulates the expression of lipogenic genes via changed LXRα expression in vivo. GH increased SCD-1 mRNA and protein irrespective of the mode of exposure, an effect exerted directly on the hepatocyte. Although increased SREBP-1c gene expression has been shown to be important in the regulation of several lipogenic enzymes, our data show that increased SREBP-1c gene expression is not responsible for the effect of the female GH secretory pattern on FAS and GPAT mRNA or the effect of GH on SCD-1 mRNA. An alternative transcriptional regulation of these genes by GH could be via changed expression of upstream stimulatory factor (USF)-1, which is upregulated by insulin and binds to E-box element in the FAS promoter (51). Interestingly, a low dose of GH given as a continuous infusion to intact male rats has been shown to increase USF-1 mRNA expression in the liver (16), indicating that increased USF-1 expression could mediate the effect of continuous GH secretion on FAS transcription.

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