Direct effects of growth hormone on production and secretion of apolipoprotein B from rat hepatocytes

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Direct effects of growth hormone on production and secretion of apolipoprotein B from rat hepatocytes. Am J Physiol Endocrinol Metab 279: E1335–E1346, 2000.—The aim of this study was to investigate the direct effects of growth hormone (GH) on production and secretion of apolipoprotein B (apoB)-containing lipoproteins from hepatocytes. Bovine GH (5–500 ng/ml) was given for 1 or 3 days to rat hepatocytes cultured on laminin-rich matrigel in serum-free medium. The effects of GH were compared with those of 3 nM insulin and 500 μM oleic acid. GH increased the editing of apoB mRNA, and the proportion of newly synthesized apoB-48 (of total apoB) in the cells and secreted into the medium changed in parallel. GH increased total secretion of apoB-48 (+30%) and apoB-48 in very low-density lipoproteins (VLDL) more than twofold. Total apoB-100 secretion decreased 63%, but apoB-100-VLDL secretion was unaffected by GH. Pulse-chase studies indicated that GH increased intracellular early degradation of apoB-100 but not apoB-48. GH had no effect on apoB mRNA or LDL receptor mRNA levels. The triglyceride synthesis, the mass of triglycerides in the cells, and the VLDL fraction of the medium increased after GH incubation. Three days of insulin incubation had effects similar to those of GH. Combined incubation with oleic acid and GH had additive effects on apoB mRNA editing and apoB-48-VLDL secretion. In summary, GH has direct effects on production and secretion of apoB-containing lipoproteins, which may add to the effects of hyperinsulinemia and increased flux of fatty acids to the liver during GH treatment in vivo.

insulin; fatty acids; apolipoprotein B-48; apolipoprotein B-100; very low density lipoproteins

Several studies in growth hormone (GH)-deficient subjects (24) and hypophysectomized rats (31, 41) indicate the importance of physiological GH secretion in the regulation of serum levels of apoB-containing lipoproteins. Studies in rat indicate that GH may affect the degradation of apoB-containing lipoproteins via increased lipoprotein lipase and hepatic lipase activity (32) as well as hepatic low-density lipoprotein (LDL) receptor expression (33, 37). In addition, in vivo studies indicate that GH influences secretion of apoB-containing lipoproteins from the liver in the rat (18, 39, 40) and in humans (9).

Very low density lipoprotein (VLDL) is the precursor of circulating LDL and, to a certain extent, high-density lipoprotein (HDL). In humans, the liver secretes only apoB-100-containing VLDL, and apoB-48 secretion occurs exclusively from the intestine (21, 22, 30). ApoB-48 mRNA is formed by a posttranscriptional and enzyme-dependent deamination (C →U), referred to as the editing of apoB mRNA, which results in the formation of a stop codon (7, 36). VLDL secreted from rat hepatocytes are of two different kinds, i.e., containing either apoB-48 or apoB-100 as structural protein (21, 30). The editing of apoB mRNA in the rat liver is developmentally regulated (28, 50) and dependent on the nutritional status of the animals (2, 21, 26). During both development and refeeding of fasted rats, increased triglyceride synthesis and secretion from the liver are associated with increased apoB mRNA editing and apoB-48 secretion (2, 11, 21, 26, 28, 50).

In previous studies on the effects of GH on triglyceride synthesis and apoB and VLDL secretion, GH was administered in vivo to hypophysectomized rats (17, 18, 39, 40). Short-term cultures of hepatocytes derived from GH-treated hypophysectomized rats showed increased editing of apoB mRNA (40), triglyceride synthesis, and assembly and secretion of apoB-48-VLDL (17, 39). GH treatment of hypophysectomized rats also increased VLDL (lipid and apoprotein) accumulation in the medium during liver perfusion (18). Moreover, it was recently shown by stable isotope technique that GH therapy of GH-deficient adults increased the apoB-100-VLDL secretion rate (9). Thus it is clear that GH has a physiological role in the regulation of VLDL secretion in both rat and human. However, it is not known whether these effects of GH are direct via the GH receptor in hepatocytes or indirect via other metabolic alterations induced by the GH therapy. GH treatment induces several metabolic effects in vivo that may potentially affect the synthesis and secretion of apoB-containing lipoproteins from hepatocytes. An effect mediated via increased hepatic insulin-like
growth factor (IGF) I production is not likely, because triglyceride and apoB production in cultured hepatocytes derived from IGF-I-treated hypophysectomized rats was unchanged (41), and hepatocytes have no or few IGF-I receptors (12). Other in vivo effects of GH are changed insulin responsiveness and increased insulin secretion (14, 19, 34). Incubation of hepatocytes with insulin increased the editing of apoB mRNA, secretion of apoB-48 (45), and triglyceride synthesis (1), effects that are similar to the observed in vivo effects of GH (17, 39, 40). Therefore, changed insulin action may potentially mediate the effects of GH on hepatic production of apoB-containing lipoproteins. Another possibility is that GH, via its lipolytic and antilipogenic effect in adipose tissue (14, 19), increases the flux of fatty acids to the liver. An increased supply of fatty acids may potentially increase apoB-48-VLDL secretion (13, 27, 30).

The aims of this study were to investigate whether GH has direct effects on the production and secretion of apoB-containing lipoproteins and to compare the effects of GH with those of insulin and oleic acid.

MATERIALS AND METHODS

The Ethics Committee of Göteborg University approved this study. All chemicals used were from Sigma Chemical (St. Louis, MO) unless stated otherwise.

Hepatocyte Cultures and Hormones

Hepatocytes were prepared by a nonrecirculating collagenase perfusion through the portal vein of 200- to 300-g normal female Sprague-Dawley rats (n = 28) as described before (6). The viability was ~90%, as determined by a Trypan blue exclusion test at the start and ~98–100% at the end of the experiments. Lactate dehydrogenase (LDH) activity was measured in the medium (BM/Hitachi 917, Boehringer Mannheim, Germany) in the experiments when oleic acid was used. In the experiments presented, no culture dishes had an increased LDH activity in the medium. The cells were seeded at a density of ~170,000 cells/cm² in plastic 100-mm dishes (Falcon, Plymouth, UK) for measurements of mRNA, triglyceride mass, and distribution of apoB in lipoprotein density classes. For measurements of total apoB-48, apoB-100, and triglyceride synthesis, 60-mm dishes (Falcon) were used. The dishes were coated with laminin-rich matrigel (Collaborative Research, Medical Products, Bedford, MA), and the cells were plated during the first 16–18 h in Williams E medium supplemented as described before (6). After 16–18 h of culture, the medium was changed to one that differed from the first medium only with respect to the content of hormones and oleic acid. The medium given during the next 3 days of culture was always supplemented with 1 nM dexamethasone. Bovine GH (a kind gift from American Cyanamide, Princeton, NJ) was given during the first 16–18 h of culture, the medium was changed to one containing hormones and oleic acid, essentially fatty acid-free bovine albumin 0.75% (wt/vol) and DMSO 0.15% (vol/vol) were added to all culture dishes. The medium was changed every day. In all experiments, the cells were cultured for 4 days.

Apob and LDL Receptor mRNA Measurements and PCR Amplification of the ApoB mRNA Editing Site

Total cellular RNA was isolated from cultured hepatocytes according to Chomczynski and Sacchi (8). A Bluescript plasmid (Stratagene, La Jolla, CA) containing a 208-bp rat apoB fragment was kindly provided by Dr. James Scott (Hammer smith Hospital, London, UK). A 236-bp fragment of the rat LDL receptor gene cDNA was cloned by PCR with the use of an oligonucleotide pair corresponding to nucleotides 1387–1407 and 1598–1623 (Scandinavian Gene Synthesis, Köping, Sweden) of the rat LDL receptor gene cDNA sequence. The PCR product was cloned into a pCR II vector (Invitrogen, San Diego, CA). The cDNA fragments of the apoB and LDL receptor genes were sequenced to confirm the identity. ApoB mRNA and LDL receptor mRNA were measured by hybridization of [32P]CTP (Amersham, Buckinghamshire, UK) antisense transcripts with total RNA in a ribonuclease protection assay. An 80-bp fragment of a highly conserved region of the 18S ribosomal gene was used as internal control (Ambion, Austin, TX). Quantification was performed with a PhosphorImager and Image Quant software (Molecular Dynamics, Sunnyvale, CA).

The apoB mRNA editing site was amplified using reverse transcriptase PCR. RNA samples were treated with DNase before cDNA was synthesized from 0.5 μg of total RNA by use of 0.25 μg random primers (Promega, Madison, WI) and avian myoblastosis virus reverse transcriptase (AMV-RT; GIBCO BRL). One quarter of the cDNA reaction was used to obtain a 206-bp DNA fragment that included the edited base by use of rat apoB-specific oligonucleotides (PCR 1, PCR 2) (15). Control PCR reactions of the samples without AMV-RT were also performed. No products could be detected, indicating that no contaminating genomic DNA was present. The DNA was amplified 25 cycles, and the PCR product obtained was analyzed by agarose gel electrophoresis to confirm the size of the amplified product and was subsequently purified from the gel on a microspin column (Qiagen, Germany) before primer extension analysis.

Primer Extension Analysis

To analyze the extent of apoB mRNA editing, primer extension was performed (40). Denatured apoB PCR product (10–20 ng) was annealed overnight at 45°C to a 32P-5’-end-labeled rat apoB oligonucleotide (2). The primer was labeled with a 5’-end-labeling kit and 50 μCi of [32P]ATP (Amer sham). The gel bands corresponding to the edited and non-edited apoB mRNA were cut out of the gel, and the radioactive activity was counted after digestion of the gel (49). The intra- and interassay coefficients of variation were <3%.

DNA Concentration Analysis

A 200-μl cell suspension from each culture dish was treated with proteinase K (10 μg) at 45°C overnight and centrifuged at 10,000 g for 5 min. The DNA content in the supernatant was determined according to the method of Labarca and Paigen (25).

Estimation of Amount of Newly Synthesized apoB-48 and apoB-100

The amounts of newly synthesized apoB-48 and apoB-100 in the cells were estimated after a 2.5-h labeling with [35S]methionine-cysteine mix (Amersham) (39, 40). The secretion of newly synthesized apoB-48 and apoB-100 was estimated by labeling the cells for 2.5 h followed by a 4-h chase period with culture medium supplemented with 10 mM
methionine (39, 40). Pulse labeling of the cells for shorter periods was carried out after a 1-h preincubation in Eagle’s minimum essential medium without methionine (39). In all experiments, the cell cultures were incubated with hormones and oleic acid also during pulse labeling and the chase period. The harvested cells and medium were treated with a protease inhibitor cocktail including 0.1 mM leupeptin (ICN Biomedicals), 1 mM phenylmethylsulfonyl fluoride, 100 mM cal-cium ionophore A23187, 100 mM N-acetyl-Leu-Leu-nor-
leucinal and 0.5 mM EDTA. Labeled apoB-48 and apoB-100 were isolated by immunoprecipitation (40, 49) by use of polyclonal rabbit anti-rat apoB serum (31), followed by electrophoresis in 5 or 3–15% polyacrylamide gels containing SDS. The bands corresponding to apoB-48 and apoB-100 were cut out of the gels, and the radioactivity was counted after digestion of the gels (40, 49). The total amount of TCA-precipitable 35S-labeled protein in the cells was estimated using standard protocol. The radioactivity recovered from each culture dish was related to the DNA content of the cells in the culture dish.

Sucrose Gradient Ultracentrifugation

Gradient 1. ApoB-48- and apoB-100-containing lipoproteins accumulated in the medium were analyzed by sucrose-gradient ultracentrifugation as described (39). In brief, the sucrose was dissolved in PBS, pH 7.3. The gradient was formed by layering from the bottom of the tube: 2.0 ml 49% sucrose, 2.0 ml 25% sucrose, 5 ml of the sample in 10% sucrose, and 3.5 ml PBS (12.5 ml centrifuge tubes, no. 331374, Beckman). All solutions contained the protease inhibitors described above. The gradients were centrifuged at 35,000 rpm in a Beckman SW 40 rotor for 65 h at 12°C with slow acceleration and no brake. The gradients were unloaded from the bottom into 11 fractions. The sucrose content in each of the 11 fractions was measured with a refractometer.

Gradient 2. ApoB-48- and apoB-100-containing VLDL secreted into the medium were also analyzed by a quicker and simpler sucrose gradient. This gradient was formed by layering from the bottom of the tube: 2.0 ml 49% sucrose, 5 ml of the sample in 11% sucrose, and 6 ml of PBS in 13.2-ml Quick-Seal vials (no. 344322, Beckman). All solutions contained the same protease inhibitor cocktail as used in gradient 1. The gradients were centrifuged at 40,000 rpm in a Beckman Ti50 for 20 h at 12°C with slow acceleration and no brake. In initial experiments, the gradient was unloaded from the top of the vial in ~1-ml fractions. From the density profile of the gradient, VLDL was found in the top 3–4 ml. In the gradient, all the triglycerides (measured by an enzymatic colorimetric method) were found in the top 3.5 ml of the gradient. In subsequent experiments, the gradients were unloaded from the top into two fractions, i.e., VLDL (top 3.5 ml) and denser particles (the rest of the gradient). The mean density in the VLDL fraction was 1.009 g/ml, and the mean density in the rest of the gradient was 1.081 g/ml. The amount of labeled apoB-48 and apoB-100 recovered in the VLDL fraction (top 3.5 ml) and the percentages of apoB-48 and apoB-100 found in VLDL (of total apoB-48 and apoB-100 in the gradient) were similar after 20 and 65 h of centrifugation (data not shown).

Triglyceride Synthesis

Triglyceride synthesis was estimated by measurement of incorporation of [14C]oleic acid or [3H]glycerol in the triglyceride fraction of the cells (39, 48). Hepatocytes were incubated for 15, 30, and 60 min in 1.5 ml medium containing 1.9 μCi [14C]oleic acid (Amersham) and 500 μM oleic acid complexed to essentially fatty acid-free bovine serum albumin (40 μg/ml) (39, 48). Alternatively, the cells were incubated for 15 and 30 min in 1.5 ml medium containing 25 μCi [3H]glycerol (Amersham) with or without the addition of 500 μM glycerol (39). Triglycerides were extracted according to the method of Bligh and Dyer (4) and were subjected to thin-layer chromatography on a silica gel 60 (Merck), first in chloroform-
methanol-H2O (65:25:4 vol/vol/vol) and then in petroleum ether-ether-acetic acid (80:20:1, vol/vol/vol). The bands were visualized with iodine vapor. Triglycerides were extracted from the silica gel with 0.5 ml of cyclohexane, and 10 ml of scintillation solution (Ready-Safe, Amersham) were added before the radioactivity was counted. The mass of triglycerides in the cells and the VLDL fraction of the medium (d. < 1.006 g/ml) were determined after extraction of total lipids according to Bligh and Dyer. The lipids were solubilized in isopropanol, and triglycerides were determined with an enzymatic colorimetric assay (GPO-PAP kit, Boehringer Mannheim). The radioactivity of the triglyceride fraction and the mass of triglycerides were related to the DNA content of the cells in the culture dishes.

Statistical Analysis

Values are expressed as means ± SD. Comparisons between two groups were performed with unpaired Student’s t-test. Comparisons between more than two groups were made by one- or two-way ANOVA followed by the Student-Newman-Keuls multiple range test (post hoc test) between individual groups. Individual representative experiments or pooled data from two to four experiments (different rat liver perfusions) are given. Two-way ANOVA was used to pool data, with the use of treatment and experiment as factors in the calculation. The values were transformed to logarithms when appropriate.

RESULTS

ApoB mRNA Editing

We used 1 nM dexamethasone in all cell cultures, because this dose of dexamethasone was shown in an initial experiment (3 culture dishes/group) to have a permissive role for the action of GH on the degree of apoB mRNA editing. Thus 1 nM dexamethasone had no effect alone (control, 51.1 ± 1.3%; 1 nM dexamethasone, 53.7 ± 1.3%), but it enhanced the effect of GH on editing of apoB mRNA (GH, 53.3 ± 1.8%; GH + 1 nM dexamethasone, 62.3 ± 0.6%, P < 0.05 vs. all other groups).

The effect of 3 days of incubation with different doses (5, 20, 100, and 500 ng/ml) of GH on apoB mRNA editing was studied (Fig. 1A). GH increased the editing of apoB mRNA in a dose-dependent manner (Fig. 1A). The relation between nonedited and edited apoB mRNA changed from 1:1.04 in the control group to 1:1.8 in the group given the highest dose of GH. Next, the effect of GH (100 ng/ml) on apoB mRNA editing was investigated when the hormone was given during the last 24 h of culture. The relation between nonedited and edited apoB mRNA changed from 1:1.04 in the control group to 1:1.8 in the group given the highest dose of GH. Incubation with insulin (3 nM) for 3 days increased editing of apoB mRNA from 1:0.8 to 1:1.2 by GH (Fig. 1B). Incubation with insulin (3 nM) for 3 days increased editing of apoB mRNA from 1:0.8 to 1:1.2 by GH (Fig. 1B).
whether GH has a different effect in the presence of insulin, 3 nM insulin was given during the last 3 days and GH (100 ng/ml) during the last 24 h. GH increased the editing of apoB mRNA to a similar degree in the presence of insulin (Fig. 1C). The relation between nonedited and edited apoB mRNA changed from 1:1.3 to 1:2.1 by GH during concomitant incubation with insulin. Thus insulin and GH have similar and additive effects on apoB mRNA editing.

Intracellular Amounts and Secretion of apoB-48 and apoB-100

The in vitro effect of GH on editing of apoB mRNA indicated that GH has a direct effect on the production of apoB-48 and apoB-100. In the next series of experiments, the effect of GH on production and secretion of apoB-48 and apoB-100 was investigated. In these experiments, GH was given during the last 24 h or 3 days of culture. Incubation with GH for 24 h had similar effects but of lower magnitude than incubation with GH for 3 days (data not shown). The results of 3 days of incubation are presented (Tables 1 and 2). GH had a small effect (+26%) on the intracellular pool of labeled apoB-48 in the absence of insulin and no significant effect in the presence of insulin (Table 1). The intracellular pool of labeled apoB-100 decreased 44% after GH incubation. This effect of GH was less pronounced in the presence of insulin (Table 1). Insulin increased the intracellular pool of apoB-48 (+67%) and had no effect on the pool size of apoB-100 in the cells. The proportion of apoB-48 (of the total labeled pool of apoB in the cells) increased by GH in the absence and in the presence of insulin during the last 3 days of culture. Cells were labeled for 2.5 h with a [35S]methionine-cysteine mix. Amounts of labeled apoB-48 and apoB-100 in the cells were measured as described in MATERIALS AND METHODS. Proportion of apoB-48 (%total apoB in the cells) was calculated as apoB-48/(apoB-48 + apoB-100). Results are pooled data from 4 different experiments with 3–4 culture dishes/group. Mean value of the control group (cell cultures given 1 nM dexamethasone alone) in each experiment is given as 100%. Values with different superscripts are significantly different from each other (P < 0.05, two-way ANOVA followed by Student-Newman-Keuls test).

Table 1. Effects of GH and insulin on the intracellular amount of newly synthesized apoB-48 and apoB-100

<table>
<thead>
<tr>
<th>Group</th>
<th>ApoB-48 (％control)</th>
<th>ApoB-100 (％control)</th>
<th>Proportion of apoB-48 (%total apoB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 16§</td>
<td>100 ± 17*</td>
<td>67.8 ± 4.7‡</td>
</tr>
<tr>
<td>GH</td>
<td>126 ± 30*</td>
<td>56 ± 22‡</td>
<td>82.8 ± 4.3*</td>
</tr>
<tr>
<td>Insulin</td>
<td>167 ± 45‡</td>
<td>109 ± 26*</td>
<td>78.2 ± 1.3†</td>
</tr>
<tr>
<td>GH + Insulin</td>
<td>184 ± 43*</td>
<td>81 ± 29†</td>
<td>84.6 ± 2.9*</td>
</tr>
</tbody>
</table>

Values are means ± SD. GH, growth hormone; apoB, apolipoprotein B. After plating, the cells were given 1 nM dexamethasone and either GH (100 ng/ml), 3 nM insulin, or a combination of GH and insulin during the last 3 days of culture. Cells were labeled for 2.5 h with a [35S]methionine-cysteine mix. Amounts of labeled apoB-48 and apoB-100 in the cells were measured as described in MATERIALS AND METHODS. Proportion of apoB-48 (%total apoB in the cells) was calculated as apoB-48/(apoB-48 + apoB-100). Results are pooled data from 4 different experiments with 3–4 culture dishes/group. Mean value of the control group (cell cultures given 1 nM dexamethasone alone) in each experiment is given as 100%. Values with different superscripts are significantly different from each other (P < 0.05, two-way ANOVA followed by Student-Newman-Keuls test).

Table 2. Effect of GH and insulin on the accumulation of newly synthesized apoB-48 and apoB-100 in the medium

<table>
<thead>
<tr>
<th>Group</th>
<th>ApoB-48 (％control)</th>
<th>ApoB-100 (％control)</th>
<th>Proportion of apoB-48 (%total apoB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 10§</td>
<td>100 ± 11*</td>
<td>52.7 ± 10.2§</td>
</tr>
<tr>
<td>GH</td>
<td>132 ± 38*</td>
<td>37 ± 16*</td>
<td>81.2 ± 7.8*</td>
</tr>
<tr>
<td>Insulin</td>
<td>193 ± 85*</td>
<td>71 ± 24*</td>
<td>75.5 ± 8.5*</td>
</tr>
<tr>
<td>GH + Insulin</td>
<td>207 ± 94*</td>
<td>40 ± 14*</td>
<td>85.3 ± 5.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD. The experimental situation was the same as in Table 1, but the cells were washed and cultured for 4 h in medium containing 10 mM unlabeled methionine after 2.5 h of pulse labeling. Amounts of labeled apoB-48 and apoB-100 in the medium were measured as described in MATERIALS AND METHODS. Proportion of apoB-48 was calculated as apoB-48/(apoB-48 + apoB-100). Results are pooled data from 4 different experiments with 3–4 culture dishes/group. Mean value of the control group (cell cultures given 1 nM dexamethasone alone) in each experiment is given as 100%. Values with different superscripts are significantly different from each other (P < 0.05, two-way ANOVA followed by Student-Newman-Keuls test).
insulin in the culture medium (Table 1). Thus the changed proportion of apoB-48 in the cells paralleled the changed editing of apoB mRNA (compare with Fig. 1, B and C).

The secretion of apoB-48 and apoB-100 was estimated by 2.5-h pulse labeling followed by 4-h incubation in a medium containing 10 mM unlabeled methionine (Fig. 2 and Table 2). Initial experiments showed a slower accumulation of newly synthesized apoB-48 than of apoB-100 in the medium (Fig. 2). GH incubation decreased the accumulation of newly synthesized apoB-100 in the medium, which was evident after 20 min of chase. The effect of GH on apoB-48 accumulation in the medium was observed later during the chase period (Fig. 2).

Incubation with GH increased labeled apoB-48 in the medium after a 4-h chase period with 32%, but GH had no significant effect in the presence of insulin (Table 2). The amount of labeled apoB-100 in the medium decreased after incubation with GH in the absence and in the presence of insulin (Table 2). Insulin increased the amount of labeled apoB-48 and decreased the amount of labeled apoB-100 in the medium (Table 2). The proportion of apoB-48 (of the total labeled pool of apoB in the medium) increased by GH (Table 2).

In three of the experiments presented above, the effect of GH on the disappearance of newly synthesized apoB-48 and apoB-100 from the cell cultures during the 4-h chase period was calculated (Fig. 3). The disappearance of apoB-48 and apoB-100 was calculated as the amount of labeled protein left in the system (cells and medium) after the 4-h chase period in relation to the amount of labeled protein in the cells after the 2.5-h labeling period. GH had no effect on the disappearance of apoB-48, but it increased the disappearance of apoB-100 during the chase period (Fig. 3). For comparison, incubations with insulin alone resulted in a decreased disappearance of apoB-48 (13 ± 9%, n = 2) and an increased disappearance of apoB-100 from the

![Fig. 2. Effects of GH on the accumulation of newly synthesized apoB-48 (A) and apoB-100 (B) in medium. After all dishes were plated with 16 nM insulin during the first 16–18 h, the cells were given 1 nM dexamethasone and cultured in absence (○) or presence of GH (100 ng/ml, ■) during the last 3 days of culture. Cells were labeled for 2.5 h with a [35S]methionine-cysteine mix, washed, and cultured for 0, 20, 40, 60, 120, or 240 min in medium containing 10 mM unlabeled methionine (chase medium). Amounts of labeled apoB-48 and apoB-100 in the chase medium were measured as described in MATERIALS AND METHODS. Results are from 1 experiment with 3 culture dishes/group. Values are means ± SD.](http://ajpendo.physiology.org/)

![Fig. 3. In 3 of the experiments presented in Tables 1 and 2, the effect of GH on the disappearance of apoB-48 and apoB-100 from the system was calculated. The disappearance of apoB-48 and apoB-100 was calculated as the amount of labeled protein left in the system (cells and medium) after the 4-h chase period in relation to the amount of labeled protein in the cells after the 2.5-h labeling period. Each observation is the mean value from 3 different experiments. Results presented are means ± SD. Values with different superscripts are significantly different from each other (P < 0.05, Student’s t-test).](http://ajpendo.physiology.org/)
system (32 ± 13%, n = 2) compared with control cultures. After the 4-h chase period, no labeled apoB-100 was detected in the cells after any of the hormonal incubations. The amount of apoB-48 retained in the cells after the 4-h chase period was ~10% of the labeled pool recovered in the cells after 2.5-h labeling, irrespective of hormonal incubation (data not shown).

To determine the effect of GH on synthesis and early degradation of apoB-48 and -100, hepatocytes were incubated with or without GH (100 ng/ml) for 3 days. After 1 h of preincubation in a medium lacking methionine, the cells were pulsed for 15 min using the same medium and chased for 15 min with an excess of cold methionine followed by immunoprecipitation of apoB from the cells (Fig. 4). GH increased the synthesis of apoB-48 but had no effect on the synthesis of apoB-100, as indicated by the labeling of the proteins after the 15-min pulse period. However, GH decreased the labeling of apoB-100 in the cells after 15-min chase, indicating an increased early intracellular degradation of apoB-100 (Fig. 4B).

ApoB mRNA and LDL Receptor mRNA

Because changed posttranslational degradation of apoB, especially apoB-100, may occur as a result of marked changes in the expression of LDL receptors (47), the effect of GH on the LDL receptor mRNA was determined. The effect of GH on total apoB mRNA levels was also determined (Table 3). The cells were incubated in 1 nM dexamethasone with or without GH (100 ng/ml) during the last 24 h of culture. The ratio of the apoB or LDLr band to the 18S band was calculated. The mean value of the control group (cells given 1 nM dexamethasone alone) in each experiment was given as 100%. The apoB mRNA data are from 2 different experiments with 2–3 culture dishes/group, and the LDLr mRNA data are from 3 different experiments with 3 culture dishes/group. Data were analyzed with Student’s t-test (apoB) and two-way ANOVA (LDLr).

Triglyceride Synthesis

The increased editing of apoB mRNA and changed proportion of apoB-48 (of total apoB) in the cells and medium after GH incubation suggested that GH has an in vitro effect on triglyceride synthesis (2, 11, 21, 26, 39, 40, 50). Experiments showed that the incorporation of [14C]oleic acid into cellular triglycerides was linear from 15 to 60 min of incubation in all experimental groups used in Fig. 5B (data not shown). The triglyceride synthesis was therefore estimated by measuring the incorporation of [14C]oleic acid into the triglyceride fraction of the cells after 60 min of incubation. Three days of GH incubation increased the incorporation of [14C]oleic acid into cellular triglycerides in a dose-dependent manner (Fig. 5A). The effect of 3 days of incubation with GH in the absence and presence of insulin on the incorporation of [14C]oleic acid into cellular triglycerides was investigated (Fig. 5B). GH increased the triglyceride synthesis in both the absence and the presence of insulin (Fig. 5B). Similar results were obtained when GH was given the last 24 h of culture (data not shown).

Table 3. Effect of GH on apoB mRNA and LDL receptor mRNA

<table>
<thead>
<tr>
<th>Group</th>
<th>ApoB/18S</th>
<th>LDLr/18S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 25</td>
<td>100 ± 18</td>
</tr>
<tr>
<td>GH</td>
<td>99 ± 25</td>
<td>114 ± 29</td>
</tr>
</tbody>
</table>

Values are means ± SD. The amounts of apoB mRNA and low-density lipoprotein receptor (LDLr) mRNA were determined using ribonuclease protection assay as described in MATERIALS AND METHODS. Cells were incubated in 1 nM dexamethasone with or without GH (100 ng/ml) during the last 24 h of culture. The ratio of the apoB or LDLr band to the 18S band was calculated. The mean value of the control group (cells given 1 nM dexamethasone alone) in each experiment was given as 100%. The apoB mRNA data are from 2 different experiments with 2–3 culture dishes/group, and the LDLr mRNA data are from 3 different experiments with 3 culture dishes/group. Data were analyzed with Student’s t-test (apoB) and two-way ANOVA (LDLr).
GH also increased the incorporation of [3H]glycerol into cellular triglycerides (Fig. 6). GH had a similar effect (150%) when the cells were incubated with [3H]glycerol alone and when they were incubated with [3H]glycerol together with 500 μM glycerol (Fig. 6, A and B), indicating that isotope dilution could not explain the results. In addition, the mass of triglycerides in the cells and the VLDL fraction of overnight culture medium (24-h incubation) were determined after 3 days of GH incubation (Table 4). GH increased the total amount of triglycerides both in the cells (37%) and in the VLDL fraction (67%) of the medium (Table 4).

Size Distribution of apoB-48- and apoB-100-Containing Lipoproteins in Medium

To determine the effect of GH on the distribution of apoB-48 and apoB-100 in lipoprotein fractions in the chase medium, rat hepatocytes were given dexamethasone alone or in combination with GH (100 ng/ml) during the last 3 days of culture. The cells were labeled and chased as described above. The lipoprotein fractions were isolated by sucrose gradient ultracentrifugation with gradient 1 (see MATERIALS AND METHODS). In Fig. 7, the mean values of two experiments are shown.

Table 4. Effect of GH on total triglyceride mass in the cells and VLDL fraction of the medium

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglycerides in Cells</th>
<th>VLDL Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.3 ± 1.5†</td>
<td>2.4 ± 0.6†</td>
</tr>
<tr>
<td>GH</td>
<td>15.5 ± 5.6*</td>
<td>3.9 ± 1.4*</td>
</tr>
</tbody>
</table>

Values are means ± SD in nmol/μg DNA. VLDL, very low density lipoprotein. Cells were incubated in 1 nM dexamethasone with or without GH (100 ng/ml) during the last 3 days of culture. Total amount of triglycerides in the cells and in the VLDL fraction of the medium after 24-h incubation was determined as described in MATERIALS AND METHODS. Data are from 2 different experiments with 3 culture dishes/group. Values with different superscripts are significantly different from each other (P < 0.05, two-way ANOVA).
In the control group, almost all of the labeled apoB-48 occurred in denser particles, banding in the HDL density region (Fig. 7A). Incubation with GH resulted in an increased amount of labeled apoB-48 in the VLDL fraction (fraction 11; Fig. 7B). The increase in apoB-48-VLDL (fraction 11) was 8-fold in one experiment and 12.9-fold in the other experiment. ApoB-100 appeared in the VLDL fraction but also in the HDL and LDL density ranges of the gradient in the control incubations (Fig. 7A). After incubation with GH, very low levels of apoB-100 appeared in fractions other than the VLDL fraction, which was largely unaffected by GH (Fig. 7B). After GH incubation, the amount of labeled apoB-100 in the VLDL fraction was 65 and 74%, respectively, of the amount in the control groups in the two experiments.

Because GH increased the synthesis of triglycerides from oleic acid, the interaction between GH and oleic acid on apoB-VLDL production was investigated. The cells were labeled and chased as described before, but the lipoprotein fractions were separated into VLDL and denser lipoprotein particles by use of another sucrose gradient (gradient 2; see MATERIALS AND METHODS), which allows faster separation of the VLDL fraction (Fig. 8). Initial experiments showed that 24-h incubation with 500 μM oleic acid resulted in a small (~40%) increase in apoB-48-VLDL in the medium, whereas apoB-100-VLDL was largely unaffected (data not shown). However, 3 days of incubation with oleic acid resulted in a marked increase in apoB-48-VLDL and a

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**Fig. 7.** Effects of GH on accumulation of newly synthesized apoB-48- (○) and apoB-100-containing lipoproteins (■) in culture medium. Representative autoradiograms from 1 experiment are also shown. After plating, the cells were given 1 nM dexamethasone alone (A) or in combination with GH (100 ng/ml, B) during the last 3 days of culture. Cells were labeled for 2.5 h with a [35S]methionine-cysteine mix. Thereafter, cells were washed and cultured for another 4 h in medium containing 10 mM unlabeled methionine. The newly synthesized apoB-containing lipoproteins secreted into the medium were separated by gradient ultracentrifugation (gradient 1). The amounts of apoB-48 and apoB-100 were measured in each fraction as described in MATERIALS AND METHODS. Values in (A) and (B) are means from 2 different experiments with 1 culture dish/group. From the density profile of the 4 gradients the different lipoprotein classes are indicated.

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**Fig. 8.** Effects of GH and oleic acid on the accumulation of apoB-48- (A) and apoB-100-containing VLDL (B) in the medium. After plating, all cell cultures were given 1 nM dexamethasone and 0.75% albumin. Oleic acid (500 μM) was given the last 3 days and GH (100 ng/ml) the last 24 h of culture. Cells were labeled for 2.5 h with a [35S]methionine-cysteine mix. Thereafter, cells were washed and cultured for another 4 h in medium containing 10 mM unlabeled methionine. The newly synthesized apoB-containing lipoproteins secreted into the medium were separated by gradient ultracentrifugation (gradient 2). The amounts of apoB-48 and apoB-100 were measured in each fraction as described in MATERIALS AND METHODS. Values in (A) and (B) are means from 2 different experiments with 1 culture dish/group. Mean value of the control group (cell cultures given dexamethasone and albumin alone) is given as 100%. Values are means ± SD. Values with different superscripts are significantly different from each other (P < 0.05, two-way ANOVA followed by Student-Newman-Keuls test).
smaller increase in apoB-100-VLDL. In the experiments presented, the cells were incubated with 500 μM oleic acid during the last 3 days and with GH (100 ng/ml) during the last 24 h. With the use of gradient 2, GH incubation resulted in a 2.2-fold increase in the accumulation of apoB-48-VLDL in the medium but had no effect on the accumulation of apoB-100-VLDL (Table 5). Oleic acid incubation for 3 days resulted in a 10-fold increase in apoB-48-VLDL and a 2.1-fold increase in apoB-100-VLDL. The combined incubation with oleic acid and GH resulted in a 16-fold increase in apoB-48-VLDL, whereas apoB-100-VLDL accumulation in the medium decreased slightly compared with the effect of oleic acid alone (Fig. 8). Combination of the results presented in Figs. 7 and 8 indicates a 6.3 ± 2.0-fold (range, 2.1 to 12.9-fold) increase in apoB-48-VLDL (P < 0.05) and no effect on apoB-100-VLDL (0.9 ± 0.2-fold, range, 0.65 to 1.13-fold) as a result of GH incubation. In the same experiments as presented in Fig. 8, apoB-48 and apoB-100 were measured in an aliquot of the chase medium before the remainder of the medium was subjected to sucrose gradient ultracentrifugation (Table 5). The accumulation of apoB-48 increased and that of apoB-100 decreased in the medium after both GH and oleic acid incubations. Oleic acid increased apoB mRNA editing both when given alone and when given in combination with GH. The proportion of apoB-48 in the medium changed in parallel (Table 5). The amount of total TCA-precipitable 35S-labeled protein in the cells was not influenced by GH or oleic acid but increased by combined GH and oleic acid incubation (Table 5).

**DISCUSSION**

This study shows for the first time that GH has direct effects on production of apoB-containing lipoproteins in hepatocytes, including increased apoB mRNA editing, triglyceride synthesis, cellular triglycerides, and accumulation of apoB-48-VLDL and VLDL triglycerides in the medium. The direct effects of GH were largely similar to those obtained in short-term cultures of hepatocytes obtained from GH-treated hypophysectomized rats (17, 39, 40). Therefore, the direct effects of GH on hepatocytes are important for the in vivo effects of GH treatment of hypophysectomized rats on hepatic production of apoB-containing lipoproteins. This finding is in contrast to the effect of thyroid hormones, which were shown to increase apoB mRNA editing in vivo (15) but had no effect in vitro (28). Oleic acid and GH had additive effects on apoB-48-VLDL accumulation in the medium, indicating that the combined action of GH and fatty acids on hepatocytes augments apoB-VLDL secretion.

We used rat hepatocytes cultured on a basement membrane matrix (matrigel) derived from extraction of the Engelbreth-Holm-Swarm sarcoma, because it allows preservation of the phenotype and normal hepatocyte function (3), including expression of a functional GH receptor for several days of culture (46). Earlier studies by us and other groups have shown a well preserved phenotype and responsiveness to GH with respect to hepatic functions, such as liver fatty acid binding protein (6), IGF-I (6, 46), P-450 enzymes (46), estrogen receptor (20), and CCAAT/enhancer binding protein-α (44) expression using matrigel and serum-free culture medium. GH incubations during the last 24 h of culture had similar effects to GH incubations for 3 days. Therefore, GH not only preserves the apoB production and triglyceride synthesis of hepatocytes in culture but also induces these changes in vitro. In all cell cultures, the effect was related to DNA, since the DNA content was linearly related to the cell count. We avoided relating the effects to protein content, because the cells were cultured on protein-rich matrigel, and small contaminations of matrigel would have affected the result of protein determinations. GH had no effect on the total protein synthesis, indicating that the results of GH incubations would not have been different if the GH effects had been related to protein content.

The GH doses used were within the physiological range, as indicated by the normal mean plasma levels of GH (50–100 ng/ml) and the mean pulse heights of GH in plasma (150–300 ng/ml) in the adult rat (23). The insulin concentration in portal blood has been shown to be ~0.3–0.4 nM in the fasting rat (29). Because the insulin concentration in portal blood in the

**Table 5. Effects of GH and oleic acid on accumulation of newly synthesized apoB-48 and apoB-100 in medium, apoB mRNA editing, and total cellular protein labeling**

<table>
<thead>
<tr>
<th>Group</th>
<th>ApoB-48 (%control)</th>
<th>ApoB-100 (%control)</th>
<th>Proportion of ApoB-48 (%total apoB)</th>
<th>ApoB mRNA Editing (%edited)</th>
<th>Total Protein Labeling (%control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 12.5 † ‡</td>
<td>100 ± 6.5 † ‡</td>
<td>47.0 ± 3.7 † ‡</td>
<td>47.5 ± 0.7 †</td>
<td>100 ± 8.3 † ‡</td>
</tr>
<tr>
<td>GH</td>
<td>119 ± 15.5 † ‡</td>
<td>60 ± 8.5 †</td>
<td>63.8 ± 3.2 † ‡</td>
<td>56.2 ± 1.2 † ‡</td>
<td>111 ± 26.6 † ‡</td>
</tr>
<tr>
<td>OA</td>
<td>142 ± 6.5 * †</td>
<td>70 ± 17 † ‡</td>
<td>65.1 ± 2.6 † ‡</td>
<td>51.8 ± 2.4 †</td>
<td>111 ± 11.3 † ‡</td>
</tr>
<tr>
<td>OA + GH</td>
<td>131 ± 26.4 † ‡</td>
<td>51 ± 8.3 † ‡</td>
<td>69.2 ± 4.3 † ‡</td>
<td>60.5 ± 2.9 † ‡</td>
<td>131 ± 12.5 † ‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. After plating, all cell cultures were given 1 mM dexamethasone and 0.75% albumin. GH (100 ng/ml) was given in the last 24 h and 500 μM oleic acid the last 3 days. Cells were labeled for 2.5 h with a [35S]methionine-cysteine mix. Thereafter, cells were washed and cultured for another 4 h in a medium containing 10 mM unlabeled methionine. Amounts of labeled apoB-48 and apoB-100 were measured in the chase medium, and the proportion of apoB-48 was calculated as apoB-48/(apoB-48 + apoB-100). ApoB mRNA editing was measured with primer extension analysis as described in MATERIALS AND METHODS. Total protein labeling was measured as radioactivity in TCA-precipitable [35S]-labeled protein in the cells. Results presented are pooled data from 2 different experiments with 4 culture dishes/group. Values with different superscripts are significantly different from each other (P < 0.05, two-way ANOVA followed by Student-Newman-Keuls test).
fed state is severalfold higher, the dose of insulin used (3 nM) is within or near the high physiological range. The finding that dexamethasone had a permissive action on the effect of GH on apoB mRNA editing was not surprising, because higher doses of dexamethasone (100 nM) have been shown to increase the editing of apoB mRNA and to enhance the effect of insulin on this process (28).

GH and insulin had similar effects on production of apoB-containing lipoproteins, indicating that the increased insulin secretion as a result of GH action on pancreatic β-cells (34) may add to the direct effect of GH on hepatocytes. However, GH changes not only insulin secretion but also insulin responsiveness (14, 19). Short-term incubations of insulin (12–24 h) have been shown to decrease apoB and VLDL secretion, whereas incubation for a longer period of time stimulates the secretion (21, 42, 45). The long-term stimulatory effect of insulin on apoB-VLDL secretion has been regarded as a phenomenon dependent on insulin resistance and may mimic chronic hyperinsulinemic and insulin-resistant states (21, 42, 45). Therefore, the direct effects of GH should be regarded as being similar to the effect of hyperinsulinemia and insulin-resistant states. Hyperinsulinemia and insulin resistance occur during GH treatment of GH-deficient subjects, as indicated by the finding of increased glucose, Hb A1c, and serum insulin levels (9, 24). Thus the two- to threefold-increased apoB-100-VLDL secretion rate after 3 mo of GH therapy of GH-deficient subjects (9) may be due to both changed insulin action and the direct effect of GH on hepatocytes. In contrast with the human situation, we observed no change in apoB-100-VLDL secretion by GH incubation of hepatocytes. However, an increased secretion of VLDL-triglycerides in the rat is accompanied by a preferential increase in apoB-48-VLDL secretion and not apoB-100-VLDL, as shown here and by others (13, 21).

ApoB-48 production increased after incubation with GH, insulin, or oleic acid, but the magnitude of the effects differed. Also, the accumulation of apoB-100 in the medium decreased as a result of incubation with GH, insulin, or oleic acid. However, in contrast to insulin and oleic acid, GH reduced apoB-100 secretion markedly. The mechanisms behind the increased disappearance of apoB-100 from the cultures are not clear from the present results; however, the pulse-chase studies indicated that GH specifically increases the early intracellular degradation of apoB-100. This effect of GH may be due to one or several mechanisms, including effects on apoB-100 lipidation, ubiquitination, or proteasomal activity (30). A direct effect of GH on LDL receptor mRNA has been shown to parallel an increased binding to the LDL receptor in HepG2 cells (33). Moreover, the in vivo effect of estrogen on LDL receptor expression and mRNA levels was shown to be dependent on the presence of GH (37). We hypothesized that a markedly increased LDL receptor expression could have increased the degradation of apoB-100 more than that of apoB-48 in the cultured rat hepatocytes, as was shown in cultured mouse hepatocytes (47). However, GH had no effects on LDL receptor mRNA levels with these culture conditions; therefore, it is unlikely that an increased LDL receptor expression could explain the increased intracellular degradation of apoB-100 during GH incubation. The rate of incorporation of [14C]oleic acid and [3H]glycerol into cellular triglycerides was increased after GH incubation. A parallel increase in cellular triglyceride mass and VLDL triglycerides in the medium confirmed that esterification of fatty acids into triglycerides predominates after GH incubation. Thus the previously observed effect of GH treatment of hypophysectomized rats on triglyceride synthesis and secretion in short-term cultures of hepatocytes (17, 39) is a direct effect on the hepatocytes. Because insulin had an effect when triglyceride synthesis was maximally induced by GH (>20 ng/ml), insulin and GH may induce triglyceride synthesis via different mechanisms. It has been shown that GH given in vitro increases the activity of phosphatidate phosphohydrolase (35), which may be one possible mechanism for the increased triglyceride synthesis. However, insulin counteracted the effect of GH on this enzyme activity, at variance with the increased incorporation of the tracers into the triglyceride fraction after insulin incubation observed in this study and by others (1).

The present study indicates that GH may enhance fatty acid synthesis. However, GH has been shown to acutely inhibit acetyl-CoA carboxylase activity (5) and decrease fatty acid synthase mRNA levels in the liver (16). There are also in vivo studies that show that GH stimulates β-oxidation in hepatocytes (10, 17). Together, these studies indicate that the availability of fatty acids for triglyceride synthesis should be decreased during GH treatment. However, triglyceride synthesis, as well as assembly and secretion of apoB-48-containing VLDL, has been shown to be dependent on the mode of GH administration to hypophysectomized rats (39). Thus a continuous infusion of GH, mimicking the secretion of GH in the female rat, increased triglyceride synthesis and apoB-48-VLDL assembly and secretion (17, 39). Intermittent administration of GH to hypophysectomized rats, mimicking the secretion of GH in the male rat, had little or no effect on these parameters (39). In contrast, apoB-100-VLDL secretion was not affected by either mode of GH administration (39). Thus the present in vitro results resemble more the effects of GH given as a continuous infusion to hypophysectomized rats (17, 39), which is not surprising because the in vitro model results in a continuous presence of GH. Thus the present results indicate that fatty acid synthesis is affected differently by a continuous and intermittent administration of GH.

In the in vivo situation, GH probably increases the flux of fatty acids to the liver because lipogenesis decreases and lipolysis increases in adipose tissue by GH therapy (14, 19). Therefore, the possibility that the direct effects of GH and oleic acid incubations had additive effects on the accumulation of apoB in the medium was studied. We observed that 24 h of incu-
bation with oleic acid had a small effect on apoB-48-VLDL secretion, whereas 3 days of incubation resulted in a marked effect. This finding is in line with that of Coussons et al. (13), who showed a two- to threefold increase in apoB-48-VLDL secretion after 3 days of exposure of rat hepatocytes to oleic acid. We also observed a small increase in apoB mRNA editing, indicating that the previously observed increased editing by high-fat feeding (43) is mediated by fatty acids. Moreover, we showed that apoB-100-VLDL accumulation into the medium was stimulated by oleic acid incubation but to a lesser extent than apoB-48-VLDL. This effect is in line with the increased secretion of apoB-100-VLDL observed during liver perfusions with oleic acid of fasted rats (38).

In summary, GH was shown to have a direct effect on the production and secretion of apoB-containing lipoproteins, effects which were similar to those of a continuous administration of GH in vivo (39). The results indicate that, by increasing the editing of apoB mRNA and triglyceride synthesis, GH preferentially increases the secretion of triglycerides and apoB-48 in the VLDL fraction. Moreover, the present findings indicate that GH has the ability in vitro to increase the lipidation of the apoB-48 precursor in a second step of apoB-48 VLDL assembly (30, 39). Thus, except for the potential role of changed insulin action and increased flux of fatty acids to the liver for the observed in vivo effects of GH on VLDL secretion (17, 18, 39, 40), GH has direct effects that result in enhanced secretion of VLDL.

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