Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6983–6987, August 1992
Medical Sciences

Importance of growth hormone for the induction of hepatic low density lipoprotein receptors

(cholesterol/estrogen/pravastatin/simvastatin/metabolic regulation)

MATS RUDLING*†, GUNNAR NORSTEDT‡, HANS OLIVECRONA†§, EVA REIHNÉR§, JAN-ÅKE GUSTAFSSON‡, AND BO ANGELIN*†

*Metabolism Unit, Department of Medicine, §Department of Surgery, and †Molecular Nutrition Unit, Center for Nutrition and Toxicology, Novum, Karolinska Institutet at Huddinge University Hospital, S-141 86 Huddinge, Sweden; and ‡Department of Medical Nutrition and Center for Biotechnology, Novum, Karolinska Institutet at Huddinge University Hospital, S-141 57 Huddinge, Sweden

Communicated by Michael S. Brown, April 21, 1992

ABSTRACT **This investigation was undertaken to determine the possible role of growth hormone (GH) in the hormonal regulation of hepatic low density lipoprotein (LDL) receptor expression. Treatment of normal rats with estrogen (ethynylestradiol, 5 mg/kg per day) increased the number of hepatic LDL receptors, and the LDL receptor mRNA levels were increased 2.4-fold. However, when hypophysectomized rats were treated with estrogen, the hepatic LDL receptor number and the mRNA levels only increased slightly. Treatment with GH was important to restore the induction of hepatic LDL receptors in hypophysectomized estrogen-treated rats. Further, the hypocholesterolemic effect of estrogen was abolished in hypophysectomized rats, and GH reversed this effect. To assess the effect of GH in humans, hepatic LDL receptor binding activity was determined in liver biopsy specimens from gallstone patients pretreated with GH (12 international units/day) prior to operation. GH administration induced hepatic LDL receptors ≈2-fold, and this was accompanied by a 25% decrease in serum cholesterol. The LDL receptor stimulation caused by GH treatment was of similar magnitude as that observed upon 3 weeks of treatment with an established hypolipidemic drug (pravastatin or simvastatin). The data show that GH has an important role in the regulation of hepatic LDL receptors and suggest that GH secretion may be important for the control of plasma LDL levels in humans.**

The activity of low density lipoprotein (LDL) receptors in the liver constitutes a major mechanism by which dietary and hormonal agents may regulate plasma cholesterol levels (1, 2). Thus, by controlling LDL catabolism, the number of hepatic LDL receptors has a direct influence on the plasma LDL level. The molecular regulation of LDL receptors by cholesterol through end-product inhibition has been elucidated in detail (3, 4). In contrast, our understanding of hormonal regulation of hepatic LDL receptors is fragmentary.

A dramatic stimulation of the hepatic LDL receptor activity occurs during treatment with pharmacological doses of estrogen (5), and this is paralleled by a markedly increased clearance of plasma LDL concomitant with a profound decrease in plasma cholesterol (6–9). The physiological relevance of the estrogen-stimulated hepatic LDL receptor expression is unclear, but it has been shown to be coupled to an elevated LDL receptor mRNA level (10). The strong stimulatory effect of estrogens on LDL receptors in the liver *in vivo* has been difficult to reproduce in cell culture (11, 12), indicating that the mechanism for stimulation may be partly indirect. An indirect action is also supported by the previous observation that estrogen failed to decrease plasma cholesterol when given to hypophysectomized (Hx) rats (13). The induction of hepatic LDL receptors by estrogen administration is probably the most efficient treatment available, and it is therefore of major importance to understand the mechanism(s) responsible for the stimulatory effect of this hormone on hepatic LDL receptors. Since estrogen treatment may exert some of its effects through influence on growth hormone (GH) secretion (14), and since treatment with GH can alter plasma cholesterol levels (15, 16), we explored the potential role of GH in the regulation of hepatic LDL receptor expression. Our results demonstrate that GH is important for the induction of hepatic LDL receptors by estrogen and indicate that GH may play a role for the expression of liver LDL receptors in normal adult humans.

MATERIALS AND METHODS

Materials. $Na^{125}I$ (IMS 30) was obtained from Amersham. Osmotic minipumps (model 2001) were from Alza. Acrylamide, *N,N'*-methylenebisacrylamide, and SDS were from Bethesda Research Laboratories. Glycerol (ultrapure) was from United States Biochemical. Triton X-100 and CsCl (catalogue no. 757306) were from Boehringer Mannheim. Bio-Rad protein assay (no. 500-0001) and high molecular weight standards (no. 161-0303) were from Bio-Rad. Ethynylestradiol, diethyl pyrocarbonate, *N*-lauroylsarcosine, bovine serum albumin (fraction V, no. A-6003), Trizma base (no. T-1503), leupeptin (no. L-2884), phenylmethylsulfonyl fluoride (no. P-7626), 1,10-phenanthroline (no. P-9375), and L-thyroxine (T_4; no. T-2501) were from Sigma. Dexamethasone (Dex) (Decadron) was from MSD, Haarlem, The Netherlands. Guanidinium thiocyanate, glycine, $CaCl_2$, EDTA, and 2-mercaptoethanol were from Merck. Human GH (Somatonorm for rat experiments and Genotropin for human studies) was kindly provided by R. Gunnarsson, Kabi-Pharmacia, Stockholm.

Rats. Forty-eight mature male Sprague–Dawley rats were used. They were kept under standardized conditions with free access to water and commercial rat chow. Lights were on from 6 a.m. to 6 p.m. Hypophysectomy was performed on 200-g rats by the parapharyngeal approach. During the 10 days following the operation, body weight was monitored daily to verify failure of weight gain.

Groups of animals (four rats per group) received ethynylestradiol (5 mg/kg), dissolved in propylene glycol, by daily (at 11 a.m.) subcutaneous injections for 4 days. Hx rats receiving hormonal substitution were in addition given the hormones indicated below through a constant infusion from osmotic minipumps implanted subcutaneously under ether

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: β-VLDL, β-migrating very low density lipoprotein; Dex, dexamethasone; GH, growth hormone; Hx, hypophysectomized; IU, international unit(s); LDL, low density lipoproteins; T_4, L-thyroxine.

anesthesia at the start of the experiment (day 0). Under these conditions, the pumps deliver substances at a rate of 1 μl/hr for 1 week. Human GH was infused at a rate of 5 μg/hr, Dex at 0.8 μg/hr, and T_4 at 0.35 μg/hr. On day 5, after 4 days of treatment, the rats were decapitated (between 11 a.m. and noon). Trunk blood was collected and the livers were immediately frozen in liquid nitrogen and thereafter stored at −70°C until processing.

Patients. Altogether 21 patients with uncomplicated gallstone disease undergoing elective cholecystectomy were studied. Seven patients received GH [12 international units (IU)/day] administered subcutaneously as a single dose in the morning; the day prior to operation the patients received an additional injection of 12 IU at 7 p.m. Nine patients served as untreated controls. The patients who were asked to participate as recipients of GH were selected at random, but no formal matching or strict protocol for randomization was followed. All the women were postmenopausal. For comparison, data from 5 additional gallstone patients who had been pretreated with the hypolipidemic drugs pravastatin (40 mg/day) or simvastatin (20 mg/day) for 3 weeks are also presented. Informed consent was obtained prior to inclusion in the study, which had been approved by the ethical committee of Huddinge University Hospital.

Lipoproteins. Rabbit β-migrating very low density lipoprotein (β-VLDL) was isolated by ultracentrifugation of serum obtained from rabbits that had been on a cholesterol-enriched diet (1% cholesterol) for at least 3 weeks (17). Rabbit β-VLDL was iodinated (specific activity, 650–1100 cpm/ng) as described (18). Free ^{125}I was removed by gel filtration on a PD-10 column (Kabi-Pharmacia) followed by extensive dialysis against 0.15 M NaCl/0.01% (wt/vol) EDTA, pH 7.5.

Standard Procedure for Preparation of Hepatic Membranes for Ligand Blot Assay. Frozen samples (−70°C) of rat livers (0.5 g) or human livers (0.3–0.5 g) were homogenized twice for 10 sec with a Polytron (Kinematica, type PT 10/35, Kriens, Lucerne, Switzerland) at 4°C in 1 ml of 50 mM Tris·HCl, pH 7.5/2 mM $CaCl_2$/0.5% Triton X-100/1 mM of leupeptin/1 mM phenanthroline/1 mM phenylmethylsulfonyl fluoride. After quick sonication (twice for 2 sec), homogenates were subjected to 10 min of centrifugation at 4°C in a microcentrifuge followed by 10 min of ultracentrifugation at 30 psi (206.7 kPa) in a Beckman Airfuge at room temperature, using a prechilled ice-cold rotor. The final supernatant was collected and frozen in multiple aliquots at −70°C. Protein content of membranes was determined (19) using reagents and protocols supplied by Bio-Rad.

Two-Step Procedure for Preparation of Human Hepatic Membranes Used for Ligand Blot Assay. Fresh liver biopsy samples (≈1 g) were minced and homogenized in a Potter–Elvehjem homogenizer with a loosely fitting Teflon pestle in 9 volumes of 50 mM Tris·HCl, pH 7.4/0.3 M sucrose/50 mM NaCl/10 mM EDTA. After centrifugation of the homogenates at 20,000 × *g* for 15 min, at 4°C, the supernatants were spun at 100,000 × *g* for 60 min, and the resultant pellets were suspended and recentrifuged at 100,000 × *g* for an additional 60 min. Pellets were thereafter frozen at −70°C. Aliquots of frozen 100,000 × *g* pellets (0.1–0.2 g) were suspended by flushing 20 times through a 0.9 × 40-mm needle with a 1.0-ml syringe in 400 μl of buffer B and then sonicated twice for 5 sec with an A-350 G sonicator (Ultrasoning, Shipley, Yorks, England), at power setting 7, tuning 6. The sonicated suspensions were subjected to microcentrifugation followed by Airfuge ultracentrifugation according to the standard procedure described above.

SDS/PAGE Separation of Liver Membrane Proteins. Membrane proteins were separated without reduction in SDS/polyacrylamide gels (3% stacking gel, 6% separation gel) (20). Samples were adjusted with a loading buffer so that the final concentrations were 0.5% SDS, 2 mM $CaCl_2$, 0.5% Triton X-100, 10% glycerol, and 50 mM Tris·HCl (pH 6.8). Samples were electrophoresed with a Protean II gel apparatus (Bio-Rad) at a constant current of 45 mA per gel for 2.5–3.5 hr at 4°C. The separated proteins were transferred (21) onto 0.45-μm nitrocellulose filters (type BA 85, Schleicher & Schuell) with a Bio-Rad transblot cell (500 mA, 4°C for 20 hr). Molecular weight markers (human LDL and Bio-Rad high molecular weight standards) were reduced with 2-mercaptoethanol (4%) and boiled for 2 min prior to loading.

Ligand Blotting of Transferred Proteins. Nitrocellulose filters were dried at room temperature for 30 min and then immediately incubated for 60 min at room temperature in 5% bovine serum albumin/2 mM $CaCl_2$/50 mM Tris·HCl, pH 8.0. ^{125}I-labeled rabbit β-VLDL was then added (5 μg of protein per ml). After an additional 60 min of incubation, the medium was aspirated and filters were washed with 2 mM $CaCl_2$/50 mM Tris·HCl, pH 8.0, containing 0.5% bovine serum albumin (one rapid wash followed by two washes for 15 min followed by one rapid wash). After a final wash with 2 mM $CaCl_2$/50 mM Tris·HCl, pH 8.0, without albumin, filters were dried and subjected to autoradiography after alignment of molecular weight standard lanes that had been stained separately by amido black. The blots were exposed for the times indicated at −70°C on Cronex film with a Quanta III intensifying screen (DuPont).

For quantitation, the LDL receptor bands (≈130 kDa) of filters were cut out for γ-counting after their identification using superimposed transparent film. Background level was obtained by γ-counting of irrelevant filter pieces of the same size and has been subtracted from the data presented. In one experiment, the 130-kDa bands and the entire lanes were analyzed in a PhosphorImager model 200B (Molecular Dynamics, Sunnyvale, CA). Data are given as arbitrary units after subtraction of background activity measured from identical filter areas of an empty parallel lane.

Preparation of Hepatic RNA. Frozen (−70°C) liver tissue was homogenized in 4 M guanidinium thiocyanate in the presence of 1% 2-mercaptoethanol. After addition of *N*-lauroylsarcosine (0.5%), total RNA was isolated by ultracentrifugation of homogenates on CsCl (22). The RNA pellets were dissolved in diethyl pyrocarbonate-treated water, extracted with phenol/chloroform and chloroform, and precipitated with ethanol. The RNA was quantitated by assuming that 1 A_{260} unit = 37 μg of RNA per ml.

Solution Hybridization Analysis of LDL Receptor mRNA. mRNAs were quantitated by a solution hybridization titration assay (22). In brief, [α-^{35}S]CTP-labeled complementary RNA probes were hybridized with 5–150 μg of hepatic RNA at 68°C overnight. After RNase treatment of samples, hybrids were precipitated and collected on glass filters. The slopes of the linear hybridization signals were calculated by the method of least squares and compared with the slope generated from a synthetic mouse LDL receptor mRNA standard. The mRNA copy number was calculated by assuming 5.5 pg of DNA per cell and an RNA/DNA ratio of 2.7:1. The sequence used for the LDL receptor probe was the previously described mouse sequence (22) corresponding to nucleotides 1247–1308 in the human LDL receptor cDNA (23).

RESULTS AND DISCUSSION

We first wanted to establish whether normal hypophyseal function is of importance for the stimulation of hepatic LDL receptors by estrogen. To answer this question, normal and Hx rats were injected daily with ethynylestradiol (5 mg/kg of body weight). After 4 days of treatment, the animals were killed and hepatic membranes were prepared. Ligand blotting of hepatic membrane proteins showed that the binding of ^{125}I-labeled rabbit β-VLDL was markedly increased in hepatic membranes from estrogen-treated normal rats (Fig. 1).

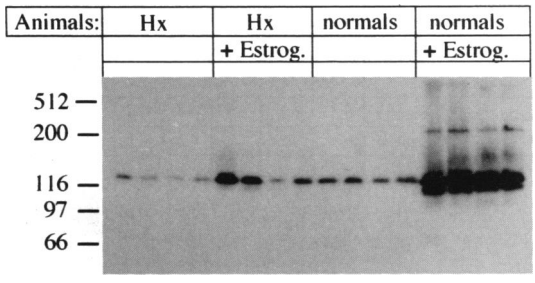

FIG. 1. Importance of the pituitary for stimulation of hepatic LDL receptors by estrogen. Normal and Hx rats were injected subcutaneously with ethynylestradiol (Estrog) or vehicle only for 4 days. On day 5, rats were killed at 11 a.m. Hepatic membranes from each animal were prepared for SDS/6% PAGE. Separated proteins (200 μg per lane) were electrotransferred to a nitrocellulose filter and incubated with ^{125}I-labeled rabbit β-VLDL. Autoradiographic exposure time was 2 hr. Molecular weight markers ($M_r \times 10^{-3}$) are at left.

The LDL receptors were visualized as a 120-kDa band; an additional band at 210–230 kDa, corresponding to the dimer of the LDL receptor (24), appeared in samples abundant in LDL receptors. However, there was little or no stimulation of hepatic LDL receptors among estrogen-treated Hx rats. Hepatic LDL receptor expression was slightly lower in untreated Hx rats than in untreated normal rats. Thus, normal hypophyseal function is a prerequisite for the pronounced stimulation of hepatic LDL receptors following estrogen administration.

To determine whether GH may be of importance for the stimulatory effect of estrogens on hepatic LDL receptors, four groups of estrogen-treated Hx rats were continuously infused with either GH alone, GH/Dex, GH/Dex/T_4, or Dex/T_4. In addition, for control purposes, four groups of rats were again treated as described in the previous experiment. After 4 days of treatment, the animals were killed and liver tissue and serum were collected. Subsequent assay of hepatic LDL receptor expression (Fig. 2*A*) in samples of pooled hepatic membranes from all eight groups demonstrated that estrogen-treated normal rats again showed a dramatic increase in LDL receptor expression; in contrast, Hx animals responded only slightly upon treatment with ethynylestradiol. However, all estrogen-treated Hx rats subjected to hormonal substitution (rat groups 5–8) showed clearly elevated hepatic LDL receptor binding activity in hepatic membranes when compared with the estrogen-treated Hx animals. Substitution with GH alone (rat group 5) resulted in an increased expression of hepatic LDL receptors. Additional substitution with Dex (group 6) gave a further increase in hepatic LDL receptor binding and substitution with GH, Dex, and T_4 in combination (group 7) revealed LDL receptor expression similar to that seen in estrogen-treated normal rats (rat group 3). However, when GH only was omitted (Dex plus T_4; group 8), the magnitude of stimulation of hepatic LDL receptors was markedly reduced.

Quantification of LDL receptor mRNA (Fig. 2*B*) revealed similar results. Estrogen treatment of normal rats (group 3) resulted in a 240% increase in LDL receptor mRNA levels as compared with normal rats, whereas estrogen-treated Hx rats showed only a 40% increase as compared with Hx rats, the latter group having mRNA levels similar to those found in normal rats. Hormonal substitution of estrogen-treated Hx rats with GH alone increased LDL receptor transcript levels by 130%. Additional substitution of estrogen-treated Hx rats with Dex or Dex/T_4 resulted in a slight further increase in LDL receptor mRNA, representing 140% and 160% increases in relation to Hx rats, respectively. However, omission of GH only (rat group 8) resulted in a lower LDL

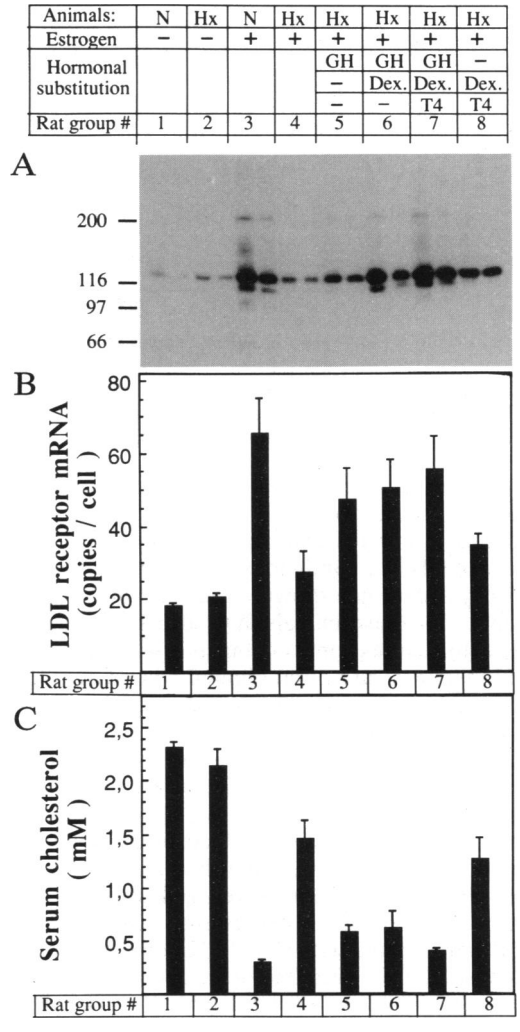

FIG. 2. Importance of GH for stimulation of hepatic LDL receptors by estrogen. Normal and Hx rats were treated as described in Fig. 1. In addition, groups of estrogen-treated Hx rats received hormonal substitution by continuous subcutaneous infusion (osmotic minipump) of the indicated hormones for 1 week. After 4 days of ethinylestradiol treatment rats were killed at 11 a.m. on day 5. (*A*) Pooled hepatic membranes were subjected to ligand blotting. Two lanes (200 and 100 μg of membrane protein, respectively) were run for each group of animals. Exposure time was 1.5 hr. (*B*) LDL receptor mRNA levels were individually quantified by solution hybridization using hepatic total RNA. (*C*) Total serum cholesterol in each individual was determined by an enzymatic method. Each group comprised four rats. Bars represent SEM.

receptor mRNA level, so that only a 70% stimulation was obtained.

Analysis of serum cholesterol (Fig. 2*C*) showed corresponding results; total serum cholesterol was thus markedly decreased upon estrogen treatment of normal rats. No difference was seen between Hx animals and normal rats, whereas a partial decrease in total cholesterol was seen in estrogen-treated Hx animals. A pronounced hypocholesterolemia was found in all groups receiving GH, the levels being in a range similar to that of normal animals treated with estrogen. However, substitution with Dex/T_4 only (group 8) resulted in serum cholesterol levels in the same range as in estrogen-treated Hx control animals (group 4). When the LDL receptor mRNA levels of the eight rat groups in Fig. 2 were plotted against the logarithm of the total serum cholesterol level in the respective groups, a strong negative correlation was obtained (Fig. 3). Thus, it could be concluded that the continuous presence of GH was necessary to obtain a

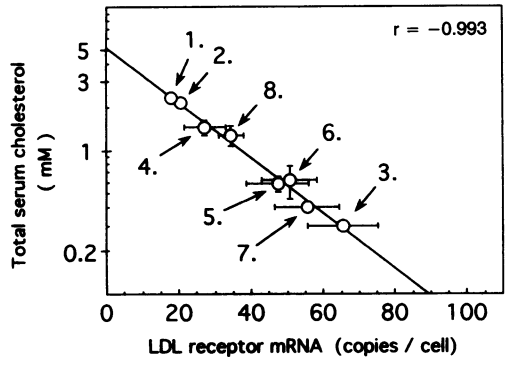

FIG. 3. Relation between hepatic LDL receptor mRNA levels and total serum cholesterol for groups 1–8 described in Fig. 2. Bars show SEM. Coefficient of correlation (r) is −0.993.

pronounced stimulation of hepatic LDL receptors upon pharmacotherapy with estrogen.

In additional control experiments, we administered the same dose of GH as a continuous infusion to normal rats (200 g) for 5 days (data not shown). Ligand blotting of hepatic membranes from these animals showed an unchanged hepatic LDL receptor expression as compared with normal control rats. GH responses are often blunted when GH is administered to normal young rats having high endogenous levels of GH. In the rat, the daily total secretion of GH is severalfold higher on a body-weight basis than in normal humans (14, 25–29). Further, GH secretion decreases progressively in humans ≥40 years of age (14, 30). These observations prompted us to study the direct effects of GH administration on hepatic LDL receptors in adult humans.

Six patients with uncomplicated gallstone disease [four men and two women; serum cholesterol, 7.2 ± 0.5 mM (mean ± SEM)] were treated with GH (12 IU/day) for 5 days prior to cholecystectomy. This treatment decreased total serum cholesterol by ≈25% (to 5.6 ± 0.5 mM; $P < 0.05$; Wilcoxon test). Solubilized liver tissue from these patients was compared with solubilized liver tissue from six untreated gallstone patients (two men and four women; serum cholesterol, 7.3 ± 0.6 mM). The human LDL receptors were visualized as a 130-kDa band, in agreement with previous observations (31). With this procedure, there was some evidence of degradation of LDL receptors and, in addition, receptor signals were relatively low compared to background. However, quantitation of the radioactivity on the filter by use of a PhosphorImager scanner showed a significantly higher activity in the 130-kDa region ($P < 0.05$, Mann–Whitney test) from the liver biopsy samples from GH-treated patients compared with untreated gallstone patients (369 ± 49 vs. 248 ± 40 arbitrary units).

To increase the assay sensitivity of human liver samples, membranes were prepared by a two-step procedure from three GH-treated patients where liver tissue was available and from an additional GH-treated man. Membrane preparations derived from these four GH-treated subjects were compared with identically isolated membranes from three additional healthy controls (all women) matched for serum cholesterol levels. This alternative membrane preparation improved the LDL receptor signals; the GH-treated subjects had 2- to 3-fold higher numbers of hepatic LDL receptors than the untreated controls (Fig. 4).

To allow a comparison of the GH-induced LDL receptor stimulation with the degree of LDL receptor stimulation observed during established pharmacotherapy (32), we compared the same three control samples with samples obtained from five patients who had received pravastatin or simvastatin for 3 weeks prior to surgery. These drugs, which are specific inhibitors of the rate-limiting enzyme in cholesterol

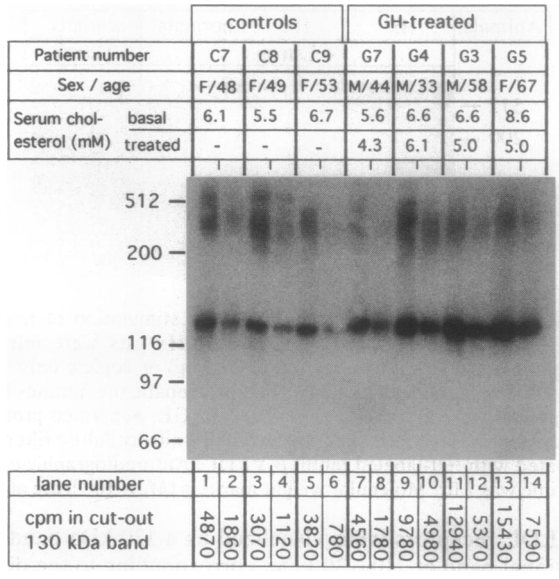

FIG. 4. Stimulation of hepatic LDL receptors by GH in humans. Liver biopsy samples were obtained from seven patients operated on for gallstone disease. Four subjects were pretreated with GH for 5 days. Liver membranes were prepared by the two-step procedure. Membrane proteins were subjected to ligand blotting after SDS/3–8% PAGE. Two concentrations of membrane protein (100 and 50 μg) from each subject were applied in odd- and even-numbered lanes, respectively. Exposure time was 2.5 hr. Total serum cholesterol (mM) was assayed in a sample obtained immediately prior to operation. ^{125}I radioactivity (cpm) was determined in the cut-out 130-kDa region.

biosynthesis, hydroxymethylglutaryl-CoA reductase, produced a similar stimulation of hepatic LDL receptors as that seen among the GH-treated subjects (Fig. 5). Thus, a clinically relevant stimulatory effect of GH on hepatic LDL receptors concomitant with a pronounced reduction of serum

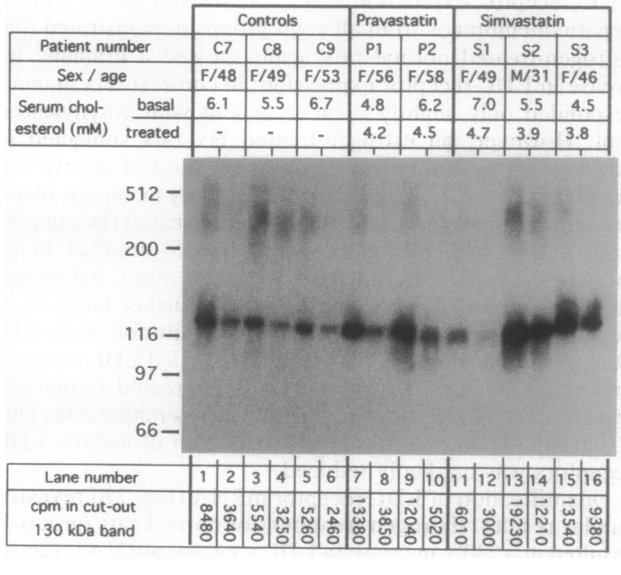

FIG. 5. Stimulation of hepatic LDL-receptors upon treatment of gallstone patients with pravastatin and simvastatin. After 3 weeks of drug treatment, cholecystectomy was performed and hepatic membranes were immediately prepared by the two-step procedure. Two concentrations of membrane protein (100 and 50 μg) from each subject were applied in odd- and even-numbered lanes, respectively. Exposure time was 2.5 hr. Total serum cholesterol was determined in a sample obtained immediately prior to operation. The three controls (C7, C8, and C9) were identical to the ones used for Fig. 4). ^{125}I radioactivity in the cut-out 130-kDa region was determined as in Fig. 4.

cholesterol levels could be demonstrated, suggesting that GH is important in the complex hormonal control of plasma LDL levels in humans.

How does GH exert its stimulatory effect on hepatic LDL receptors? GH seems to have several actions on hepatic functions (33). The sex-differentiated hepatic steroid and drug metabolism in the rat is under control of the sexually dimorphic GH secretory pattern (34–36). Estrogen-induced feminization of certain hepatic enzymes can be fully reproduced by a continuous infusion of GH, which mimics the female GH secretory pattern (34–37). GH might also modulate hepatic responsiveness to direct actions of estrogen by controlling the expression of hepatic estrogen receptors (38). Additional mechanisms may operate involving direct or indirect effects of GH on other targets of importance in hepatic cholesterol metabolism, such as cholesterol 7α-hydroxylase and hydroxymethylglutaryl-CoA reductase, the rate-limiting enzymes in the synthesis of bile acids and cholesterol, respectively. Whatever the mechanism, the role of GH—and its potential effect mediators, such as insulin-like growth factor I (33)—in the regulation of hepatic LDL receptors must now be further studied in various species for a more detailed understanding of the physiological and biochemical links between GH, estrogen, and hepatic cholesterol metabolism. Of particular interest will be to evaluate the possible relevance of the age-related reduction of GH secretion in humans (14, 30) for the decrease in plasma LDL clearance that occurs with age (39, 40).

Should adult patients with GH deficiency receive GH in addition to the current substitution regimen [thyroid hormone and corticosteroids (41)]? Hypercholesterolemia is common in such patients, and a doubling in death rate due to myocardial infarction has been reported among Hx subjects receiving conventional substitution therapy (42). Although our findings may provide some support for prolonged GH therapy, it is too early to state that additional substitution with GH to these patients may be beneficial, and a detailed evaluation of the effects on plasma LDL levels during GH substitution in Hx patients therefore seems highly warranted.

We thank Ms. Ingela Arvidsson, Ms. Tatiana Egereva, and Ms. Camilla Ejdestig for expert technical assistance and Ms. Lena Ericsson for editorial assistance. Ms. Monica Andersson, Department of Molecular Biology, Karolinska Institute, provided help with the PhosphorImager analysis. Human GH was kindly provided by Dr. Rolf Gunnarsson, Kabi-Pharmacia, Stockholm. This work was supported by the Swedish Medical Research Council (03K-8722, 03X-4793, 03X-7137, 13X-8556, 03X-6807) and the King Gustaf V and Queen Victoria Foundation.

1. Brown, M. S. & Goldstein, J. L. (1986) *Science* **232,** 34–47.
2. Myant, N. B. (1990) *Cholesterol Metabolism, LDL, and the LDL Receptor* (Academic, San Diego).
3. Smith, J. R., Osborne, T. F., Goldstein, J. L. & Brown, M. S. (1990) *J. Biol. Chem.* **265,** 2306–2310.
4. Goldstein, J. L. & Brown, M. S. (1990) *Nature (London)* **343,** 425–430.
5. Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979) *J. Biol. Chem.* **254,** 11367–11373.
6. Hay, R. V., Pottenger, L. A., Reingold, A. L., Getz, G. S. & Wissler, R. W. (1971) *Biochem. Biophys. Res. Commun.* **44,** 1471–1477.
7. Chao, Y.-S., Windler, E. E., Chen, G. C. & Havel, R. J. (1979) *J. Biol. Chem.* **254,** 11360–11366.
8. Windler, E. E., Kovanen, P. T., Chao, Y. S., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255,** 10464–10471.
9. Eriksson, M., Berglund, L., Rudling, M., Henriksson, P. & Angelin, B. (1990) *J. Clin. Invest.* **84,** 802–810.
10. Ma, P. T., Yamamoto, T., Goldstein, J. L. & Brown, M. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 792–796.
11. Semenkovich, C. F. & Ostlund, R. E. (1987) *Biochemistry* **26,** 4987–4992.
12. Wade, D. P., Knight, B. L. & Soutar, A. K. (1989) *Eur. J. Biochem.* **181,** 727–731.
13. Steinberg, M., Tolksdorf, S. & Gordon, A. S. (1967) *Endocrinology* **81,** 340–344.
14. Thorner, M. O. & Vance, M. L. (1988) *J. Clin. Invest.* **82,** 745–747.
15. Friedman, M., Byers, S. O. & Elek, S. R. (1970) *Nature (London)* **255,** 464–467.
16. Friedman, M., Byers, S. O., Rosenman, R. H., Li, C. H. & Neumann, R. (1974) *Metabolism* **23,** 905–912.
17. Henriksson, P., Stamberger, M., Eriksson, M., Rudling, M., Diczfalusy, U., Berglund, L. & Angelin, B. (1989) *Eur. J. Clin. Invest.* **19,** 395–403.
18. Langer, T., Strober, W. & Levy, R. I. (1972) *J. Clin. Invest.* **51,** 1528–1536.
19. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
20. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
21. Burnette, W. N. (1981) *Anal. Biochem.* **112,** 195–203.
22. Rudling, M. (1992) *J. Lipid Res.* **33,** 493–501.
23. Yamamoto, T., Davis, C. G., Brown, M. S., Schneider, W. J., Casey, M. L., Goldstein, J. L. & Russell, D. W. (1989) *Cell* **39,** 27–38.
24. van Driel, I. R., Davis, C. G., Goldstein, J. L. & Brown, M. S. (1987) *J. Biol. Chem.* **262,** 16127–16134.
25. Tannenbaum, G. S. & Martin, J. B. (1976) *Endocrinology* **98,** 562–570.
26. Johnson, R. J. (1988) *J. Endocrinol.* **119,** 101–109.
27. Asplin, C. M., Faria, A. C., Carlsen, E. C., Vaccaro, V. A., Barr, R. E., Iranmanesh, A., Lee, M., Veldhuis, J. D. & Evans, W. S. (1989) *J. Clin. Endocrinol. Metab.* **69,** 239–245.
28. Winer, L. M., Shaw, M. A. & Baumann, G. (1990) *J. Clin. Endocrinol. Metab.* **70,** 1678–1686.
29. Waxman, D. J., Pampori, N. A., Ram, P. A., Agrawal, A. K. & Shapiro, B. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6868–6872.
30. Rudman, D., Kutner, M. H., Rogers, C. M., Lubin, M. F., Fleming, G. A. & Bain, R. P. (1981) *J. Clin. Invest.* **67,** 1361–1369.
31. Soutar, A. K., Harders-Spengel, K., Wade, D. P. & Knight, B. L. (1986) *J. Biol. Chem.* **268,** 17127–17133.
32. Reihnér, E., Rudling, M., Ståhlberg, D., Berglund, L., Ewerth, S., Björkhem, I., Einarsson, K. & Angelin, B. (1990) *N. Engl. J. Med.* **323,** 224–228.
33. Russell, W. E. (1985) *Semin. Liver Dis.* **5,** 46–58.
34. Mode, A., Norstedt, G., Simic, B., Eneroth, P. & Gustafsson, J.-Å. (1981) *Endocrinology* **108,** 2103–2108.
35. Mode, A., Gustafsson, J.-Å., Jansson, J.-O., Edén, S. & Isaksson, O. (1982) *Endocrinology* **111,** 1692–1697.
36. Gustafsson, J.-Å., Mode, A., Norstedt, G. & Skett, P. (1983) *Annu. Rev. Physiol.* **45,** 51–60.
37. Jansson, J.-O., Edén, S. & Isaksson, O. (1985) *Endocrinol. Rev.* **6,** 128–150.
38. Norstedt, G., Wrange, Ö. & Gustafsson, J.-Å. (1981) *Endocrinology* **108,** 1190–1196.
39. Grundy, S. M., Vega, G. L. & Bilheimer, D. W. (1985) *Arteriosclerosis* **5,** 623–630.
40. Ericsson, S., Eriksson, M., Vitols, S., Einarsson, K., Berglund, L. & Angelin, B. (1991) *J. Clin. Invest.* **87,** 591–596.
41. Salomon, F., Cuneo, R. C., Hesp, R. & Sönksen, P. H. (1989) *N. Engl. J. Med.* **321,** 1797–1803.
42. Rosén, T. & Bengtsson, B.-Å. (1990) *Lancet* **336,** 285–288.