Regulation of Rat Hepatic Low Density Lipoprotein Receptors

In Vivo Stimulation by Growth Hormone Is Not Mediated by Insulin-like Growth Factor I

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Abstract

Growth hormone (GH) has an important role in the regulation of hepatic LDL receptor expression and plasma lipoprotein levels. This investigation was undertaken to evaluate if these effects of GH on hepatic LDL receptors are direct or mediated by insulin-like growth factor I (IGF-I). Two models were studied in which substitution with GH is important for the regulation of hepatic LDL receptors: hypophysectomized rats receiving high-dose ethynylestradiol or challenge with dietary cholesterol. The hypophysectomized rats were hormonally substituted by infusion with dexamethasone and L-thyroxine, and either GH or IGF-I. In both models, GH was essential for maintaining normal expression of LDL receptors. In contrast, despite fully normalized plasma levels, IGF-I did not support the expression of hepatic LDL receptors. Analysis of plasma lipoproteins revealed that substitution with GH, but not with IGF-I, reduced LDL and intermediate density lipoproteins. In addition, determination of hepatic mRNA levels for apo B-100 and apo B-48 indicated that GH may be more effective than IGF-I in the promotion of apo B mRNA editing. In conclusion, GH has specific effects on hepatic LDL receptor expression and plasma lipoprotein levels that are not mediated by IGF-I. (*J. Clin. Invest.* 1996. 97:292–299.) Key words: LDL receptor • somatotropin • IGF-I • apo B editing • hypophysectomy

Introduction

Increased levels of plasma cholesterol, particularly LDL cholesterol, are associated with an enhanced risk of atherosclerosis and coronary heart disease (1, 2). Therapeutic reduction of total and LDL cholesterol by pharmacological or surgical intervention can reduce the development of coronary atherosclerosis (3–6) and may prolong life in individuals with manifest disease (7). Great interest has therefore been paid to finding new ways of efficiently reducing plasma total and LDL cholesterol.

A major mechanism by which plasma LDL cholesterol levels may be influenced is via the regulation of hepatic LDL receptor expression (8–11). By controlling LDL catabolism, the number of hepatic LDL receptors directly governs the plasma LDL cholesterol concentrations. Thus, by pharmacological or surgical interaction with bile acid enterohepatic circulation or by inhibition of cholesterol biosynthesis in the liver, the activity of hepatic LDL receptors can be increased in animals and humans (12–15). This receptor regulation depends on end-product inhibition of LDL receptor expression by cholesterol or hydroxylated derivates thereof (8, 16).

The activity of hepatic LDL receptors is also under hormonal control (17). For example, it has been shown that estrogen, thyroid hormone, insulin, and glucagon may stimulate LDL receptor expression and lower plasma LDL cholesterol (18–23). Of particular interest is the recent demonstration that pituitary growth hormone (GH)[1] stimulates hepatic LDL receptors in humans (20). Furthermore, the presence of GH is important in obtaining the stimulatory effect of high-dose estrogen on hepatic LDL receptors (20). GH is also important in maintaining the hepatic LDL receptors resistant to suppression by dietary cholesterol in the rat (24).

In addition to its influence on hepatic LDL receptor expression, GH exerts several other effects on lipoprotein metabolism (25). Thus, GH increases lipolysis in adipose tissue and stimulates the synthesis and secretion of VLDL triglycerides by the liver (26, 27). Furthermore, GH stimulates the posttranscriptional editing of apo B mRNA in rat liver (28), a regulatory action that will enrich the triglyceride-rich VLDL with the truncated form of apo B, apo B-48, and hence drive these lipoproteins into a more rapid catabolic pathway (29). The pattern of GH secretion may also influence the hepatic secretion of apo E (30), which is an important ligand for the LDL receptor as well as for other lipoprotein receptors (31). In addition, GH stimulates the activity of hepatic and lipoprotein lipase in the rat (32).

Many of the actions of GH are mediated by the GH-dependent production of IGF-I, and, in the therapy of growth disorders, IGF-I can fully substitute for GH. In cultured cells, IGF-I has been shown to stimulate LDL receptor expression (33, 34), but it is not known if IGF-I can replace GH in the stimulation of hepatic LDL receptors in vivo. This question is of great importance both for our understanding of the role of GH in lipoprotein metabolism and for our therapeutic attempts to lower plasma LDL cholesterol. Thus, since IGF-I has a longer half-life, and since treatment with this compound has none of the negative side effects on glucose and insulin metabolism observed during GH therapy, treatment with IGF-I would appear clinically advantageous. To compare directly the effects of GH and IGF-I on hepatic LDL receptor expression and on

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1. *Abbreviations used in this paper:* β-VLDL, β-migrating VLDL; Dex, dexamethasone; FPLC, fast protein liquid chromatography; GH, growth hormone; Hx, hypophysectomized; T_4, L-thyroxin.

plasma lipoproteins, we have studied two different animal models in which GH has been shown to have distinct effects: stimulation of hepatic LDL receptors by ethynylestradiol treatment (20), and resistance to LDL receptor down-regulation by cholesterol feeding (24). Our results show that IGF-I cannot substitute for GH in these two in vivo models and thus support the concept of a direct effect of GH on hepatic LDL receptor expression.

Methods

Materials

Cholesterol (product No. C-8503) and ethynylestradiol were from Sigma Chemical Co. (St. Louis, MO). Mazola corn oil was from Corn Products Corp. Foods AB (Kristianstad, Sweden). Recombinant human GH (Genotropin) and recombinant human IGF-I were from AB Pharmacia (Stockholm, Sweden). [γ-^{32}P]ATP (5,000 Ci/mmol) was purchased from Amersham International (Little Chalfont, UK). Oligonucleotides were synthesized on a DNA synthesizer (model 380B; Applied Biosystems, Inc., Foster City, CA) and purified by HPLC (C18 reversed phase). T7 polynucleotide kinase was obtained from Boehringer Mannheim (Mannheim, Germany). Osmotic mini pumps were from Alza Corp. (models 2001 and 2NL1; Palo Alto, CA). These mini pumps deliver 1 and 10 μL solvent/h, respectively. All other materials were from previously described sources (20, 23).

Animals, Experimental Procedure

Altogether, 132 male Sprague-Dawley rats were used in four separate experiments. In every experiment shown, each animal group consisted of six animals. Rats had free access to water and chow; the light cycle hours were between 6:00 a.m. and 6:00 p.m. Hypophysectomy was performed on 7-wk-old rats by the parapharyngeal approach, and body weight was monitored to verify failure to gain weight.

At the start of each experiment, osmotic mini pumps were implanted surgically under light ether anesthesia to provide the indicated hormonal substitution. The mini pumps delivered GH at a rate of 10 μg/h (1.2 mg/kg per d). The infusion rates of IGF-I were determined from pilot substitution experiments and were set to obtain normalized plasma levels; in the experiments shown, they were 25 and 50 μg of IGF-I/h for estrogen-treated and cholesterol-fed animals, respectively. Dexamethasone (Dex) and L-thyroxin (T_4) were infused at a rate of 0.8 and 0.35 μg/h, respectively. Animals that did not receive any hormonal substitution were sham operated. Rats receiving ethynylestradiol (5 mg/ml, dissolved in propylene glycol) were given a dose of 5 mg/kg by daily subcutaneous injections at 8:00 a.m.; normal control rats received vehicle only. After 6 d of treatment, animals were killed at 10:00 a.m., 2 h after the last injection. When animals received dietary cholesterol, they were given normal rat chow supplemented with 10% corn oil into which cholesterol had been dissolved, so that the final concentration of dietary cholesterol was 2%. Rats received the diet for 6 d and were killed at 10:00 a.m.

Ligand Blot Assay of LDL Receptors. Ligand blot assay of LDL receptors was performed as described elsewhere (20). In brief, hepatic membranes were prepared from pooled liver samples of each group and separated on SDS-PAGE (6%) at nonreducing conditions, transferred to nitrocellulose filters, and incubated with ^{125}I-labeled rabbit β-migrating VLDL (β-VLDL). Pictures were generated, and 120-kD bands were quantitated in arbitrary units after background subtraction using a Bio-imaging analyzer (Fujix BAS 2000; Fuji Photo Film Co., Tokyo, Japan).

mRNA Quantitation. Hepatic total RNA was isolated by ultracentrifugation on CsCl, and the mRNA levels for the LDL receptor were quantitated by solution hybridization using a mouse cRNA probe (35). The mRNA abundance was expressed as copies of mRNA molecules per cell, assuming 15 pg of RNA per cell; this is not an absolute quantification.

Separation of Plasma Lipoproteins. Separation of plasma lipoproteins was made by column chromatography using fast protein liquid chromatography (FPLC) on a Superose-6B column (Pharmacia, Uppsala, Sweden), as detailed elsewhere (23). In brief, 4 ml of pooled plasma from all animals in each group was subjected to ultracentrifugation, and, thereafter, 1 aliquot, corresponding to 2.5 ml rat plasma, was injected onto the column. Fractions were analyzed for cholesterol and triglycerides and subjected to SDS-PAGE after precipitation with 10% TCA and subsequent delipidation with acetone.

Hepatic apo B Editing. Hepatic apo B editing was assessed as described by Baum et al. (36). In brief, a ^{32}P–end-labeled oligonucleotide of 25 bp (corresponding to nucleotides 6674–6698 in rat apo B-100) was annealed overnight at 45°C with 10 μg of pooled total RNA from each group. After precipitation, samples were subjected to primary extension using Moloney murine leukemia virus reversed transcriptase (Perkin-Elmer AB, V Frölunda, Sweden) for 90 min at 42°C in the presence of 500 mM dideoxy-GTP. Samples were loaded on a denaturing sequencing gel, and, after separation, the 33- and 38-bp radioactive bands (equivalent to apo B-48 and apo B-100) were visualized by autoradiography. The radioactivity in each band was quantitated using the Bio-imaging analyzer, and, after background subtraction, the percentage of apo B editing was calculated.

Plasma IGF-I. Plasma IGF-I was measured by radioimmunoassay using a polyclonal antibody kindly provided by Professor P. Gluckman (School of Medicine, University of Auckland, Auckland, New Zealand). The labeled IGF-I used as a tracer is a truncated variant of IGF-I with lower affinity to binding proteins. After acid ethanol extraction of the sample (37) and subsequent incubation with antibodies and tracer, the immune complex was precipitated with a second antibody in a polyethylene glycol environment and centrifuged. The pellet was counted in a gamma counter, using recombinant human IGF-I as standard. There is high cross-reactivity between rat and human IGF-I because of the high degree of homology between the peptides, whereas the assay has negligible cross-reactivity against proinsulin, insulin, and IGF-II.

Cholesterol and Triglyceride Assays. Hepatic cholesterol was extracted from liver samples as described (35). Cholesterol in plasma, FPLC fractions, and liver extracts was assayed with Nycotest Kit (Nyegaard A/S, Oslo, Norway), using a 5.2 mM cholesterol standard (product No. 14164; Merck System, Darmstadt, Germany). Plasma triglycerides were determined with a commercial kit (Boehringer Mannheim).

Statistics. Data are given as means±SEM. The significance of differences was evaluated by Student's *t*-test (38).

Results

When rats are treated with estrogen at a high dose, hepatic LDL receptors are strongly (18, 20, 23) and specifically (39) stimulated. We previously demonstrated that this stimulation is abolished in hypophysectomized (Hx) rats but reappears completely after full hormonal substitution of Hx rats with GH + Dex + T_4 in combination (20). Omission of only GH results in a reduced stimulation of LDL receptors. To evaluate if the responses obtained after full hormonal substitution could be reproduced when replacing GH with IGF-I, we substituted two groups of estrogen-treated Hx rats with GH + Dex + T_4 and IGF-I + Dex + T_4, respectively. For reference, one group of estrogen-treated Hx rats received no hormonal substitution, and another group was substituted with only Dex + T_4. To allow for comparisons with the situation in the intact rat, two groups of normal rats were given estrogen or vehicle only. After 6 d of treatment, all animals were killed and tissues were collected.

Determination of plasma IGF-I levels by radioimmunoassay, using an antibody that recognizes both rat and human

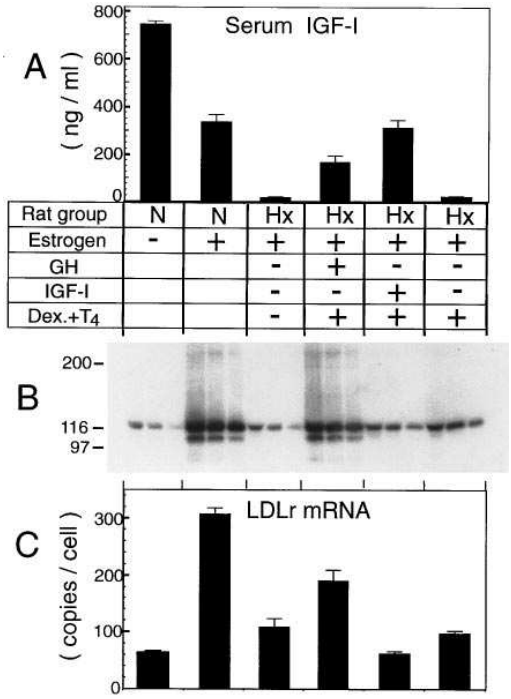

Figure 1. Different effects of GH and IGF-I in supporting the stimulation of hepatic LDL receptors by estrogen. Normal (*N*) and Hx rats were injected daily at 8:00 a.m. with ethynylestradiol subcutaneously (5 mg/kg). Three groups of estrogen-treated Hx rats received hormonal substitution by continuous subcutaneous infusion of the indicated hormones. After 6 d of treatment, rats were killed at 10:00 a.m. (*A*) Mean serum IGF-I levels determined from radioimmunoassay in individual animals. (*B*) LDL receptor determination by ligand blot. Hepatic membranes derived from pooled liver samples were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and subjected to ligand blotting using rabbit ^{125}I–β-VLDL. Three lanes were run for each group of animals (200, 100, and 50 μg of membrane protein, respectively). The picture was generated using a Bio-imaging analyzer). Data of the quantitated blot are shown in Fig. 5. (*C*) LDL receptor mRNA levels were quantitated in each individual by solution hybridization using hepatic total RNA as described. Bars represent SEM.

IGF-I, revealed that ethynylestradiol treatment of normal rats resulted in a 55% reduction, from 745±6 to 335±13 ng/ml ($P <$ 0.0001; Fig. 1 *A*). In Hx rats receiving no hormonal substitution, IGF-I levels were almost nil; the substitution of Hx rats with only Dex + T_4 did not increase IGF-I levels. However, Hx rats substituted with IGF-I + Dex + T_4 had similar IGF-I levels (314±17 ng/ml) as normal rats receiving ethynylestradiol, whereas rats receiving estrogen and GH + Dex + T_4 had clearly lower levels (166±11 ng/ml) when compared with the IGF-I–substituted group ($P < 0.01$).

Assay of hepatic LDL receptor expression in normal rats showed that ethynylestradiol treatment increased the hepatic LDL receptor expression approximately fivefold, both at the protein and at the mRNA level (Fig. 1, *B* and *C*). In contrast, ethynylestradiol treatment had only marginal effects on the LDL receptor expression in Hx animals. In line with previous results (20), substitution of Hx rats with GH + Dex + T_4 increased the LDL receptor expression, both at the protein and at the mRNA level, almost to the degree observed in normal estrogen-treated rats. In contrast, among rats substituted with IGF-I + Dex + T_4 there was little LDL receptor stimulation, and the mRNA levels for the LDL receptor were slightly reduced ($P < 0.05$) when compared with estrogen-treated Hx rats receiving Dex + T_4. The difference in hepatic LDL receptor expression between GH- and IGF-I–treated animals was confirmed from an additional blot (not shown) where individual samples were analyzed. Quantitation of LDL receptors (arbitrary units) showed that GH-treated animals had significantly higher binding (5,594±347 vs. 2,958±170; $P < 0.001$). Thus, the powerful influence of GH on the stimulatory effect of estrogen on hepatic LDL receptors could not be reproduced by the infusion of IGF-I in place of GH.

When the plasma total cholesterol levels were compared between the groups, a reciprocal pattern of response was found (Table I). Thus, a pronounced reduction (91%) was observed in normal animals after ethynylestradiol treatment, whereas only a limited effect (15% reduction) occurred in Hx rats after this treatment. Hormonal substitution of estrogen-treated Hx animals with GH + Dex + T_4 reduced plasma cholesterol levels by 60%. Among rats receiving IGF-I + Dex + T_4, plasma cholesterol was reduced by 32%, and a similar reduction was seen among animals receiving only Dex + T_4. Thus, the responses in plasma total cholesterol after substitution with GH could not be reproduced during substitution with IGF-I. A difference in the response to GH and IGF-I substitution was also evident upon comparison of plasma triglyceride levels (Table I). Furthermore, the accumulation of hepatic cholesterol was different in GH- and IGF-I–treated animals. Rats substituted with Dex + T_4 had similar hepatic total cholesterol to normal rats receiving ethynylestradiol. Among animals

Table I. Different Effects of GH and IGF-I on Cholesterol Metabolism, after Treatment of Rats with Ethynylestradiol as Described in Legend to Fig. 1

Group	N	N + E	Hx + E	Hx + E	Hx + E	Hx + E
Hormonal substitution				Dex + T_4 + GH	Dex + T_4 + IGF-I	Dex + T_4
	A	*B*	*C*	*D*	*E*	*F*
Plasma cholesterol (mM)	1.7±0.03	0.15±0.01*	1.45±0.06‡	0.58±0.02‡§‖¶	0.98±0.07‡	1.12±0.02‡
Plasma triglycerides (mM)	1.53±0.06	0.18±0.01*	0.55±0.01‡	0.27±0.02§‖¶	1.75±0.23‡§	1.45±0.10‡§
Total hepatic cholesterol (mg/g)	3.18±0.05	3.58±0.07	5.52±0.16‡	5.48±0.23‡‖¶	2.95±0.02‡§	3.47±0.11§

Plasma total cholesterol and triglycerides and hepatic cholesterol were assayed in each individual as described in Methods. Values presented are mean± SEM. Statistically significant difference ($P < 0.05$ or better) compared with *A, ‡B, §C, ‖F, ¶E. *N*, normal; *E*, estrogen.

receiving GH + Dex + T_4 substitution, hepatic cholesterol increased by 58%, whereas the hepatic cholesterol was reduced by 15% among rats receiving IGF-I + Dex + T_4.

The different effects of GH and IGF-I on plasma lipids are illustrated in more detail in Fig. 2, showing the cholesterol content in plasma lipoproteins after their separation by FPLC. After estrogen treatment of normal animals, cholesterol was virtually abolished in all lipoprotein classes (Fig. 2 *A*). Estrogen-treated Hx rats showed elevated cholesterol in all lipoprotein classes, except HDL, with a peak within the LDL range. Substitution with GH + Dex + T_4 (Fig. 2 *B*) resulted in clearly reduced intermediate density lipoprotein (IDL) and LDL cholesterol levels, whereas HDL cholesterol increased. Substitution with IGF-I + Dex + T_4 resulted in increased cholesterol content among all lipoproteins, and the FPLC pattern of this group was almost identical to that of the group receiving only Dex + T_4 (Fig. 2 *B*). Thus, in consonance with the difference in effect on hepatic LDL receptors, the effects of GH and IGF-I on plasma lipoprotein pattern were clearly different, and IGF-I could not replace GH in reducing IDL and LDL cholesterol.

We then continued to compare GH and IGF-I using a principally different rat model: cholesterol feeding of the Hx rat. In this model, we have previously demonstrated the importance of GH for the resistance to dietary cholesterol (24).

In the following experiment, the same number of animal groups and hormonal substitution regimens were used as above.

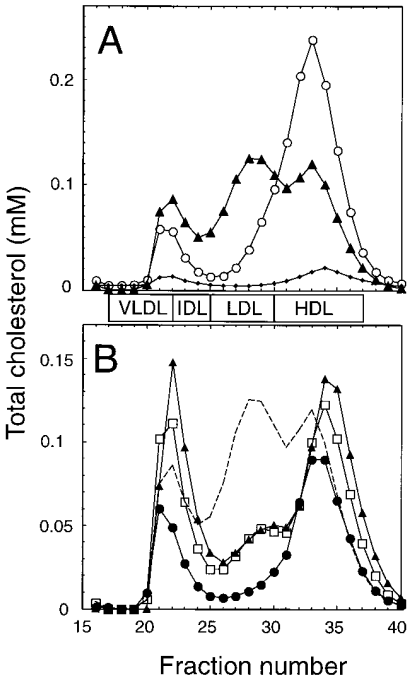

Figure 2. Plasma lipoprotein patterns after separation by FPLC of the animal groups described in the legend to Fig. 1. Blood plasma from all animals ($n = 6$) in each group was pooled (4 ml) and subjected to ultracentrifugation. 2 ml of the concentrated lipoproteins (corresponding to 2.5 ml of plasma) were then separated by FPLC as described. (*A*) Lipoprotein patterns of normal animals receiving vehicle (○) or ethynylestradiol (◆), and Hx rats receiving ethynylestradiol (▲). (*B*) Lipoprotein patterns of ethynylestradiol-treated Hx rats receiving hormonal substitution. Substitution with GH + Dex + T_4 (●), substitution with IGF-I + Dex + T_4 (□), and substitution with Dex + T_4 only (▲). For comparison, estrogen-treated Hx rats not receiving hormonal substitution from *A* are included (- - - - -).

However, instead of treating animals with ethynylestradiol, rats were challenged with 2% of dietary cholesterol. After 6 d of feeding, animals were killed and tissues were collected (Fig. 3).

Analysis of plasma IGF-I revealed that cholesterol feeding of normal rats did not alter IGF-I levels (Fig. 3 *A*). Hx rats without hormonal substitution had almost abolished IGF-I levels, and this was unaltered during substitution with Dex + T_4. Addition of IGF-I increased plasma IGF-I to the same levels (710±18 ng/ml) as found in normal animals (734±8 ng/ml), whereas IGF-I levels in rats receiving GH instead of IGF-I had increased but were still below normal IGF-I levels (229±10 ng/ml; $P < 0.001$).

As previously found (24), feeding normal rats with 2% dietary cholesterol increased hepatic LDL receptor–binding activity, in this experiment by ~ 70% (Fig. 3 *B*). This occurred although LDL receptor mRNA levels (Fig. 3 *C*) were reduced by 30% ($P < 0.0001$). Furthermore, in line with previous data (24), cholesterol feeding of Hx rats resulted in a 75% reduction of hepatic LDL receptor–binding activity compared with cholesterol-fed normal rats, and in a further reduction of LDL receptor mRNA levels ($P < 0.01$). Substitution of cholesterol-fed Hx rats with GH + Dex + T_4 almost normalized hepatic

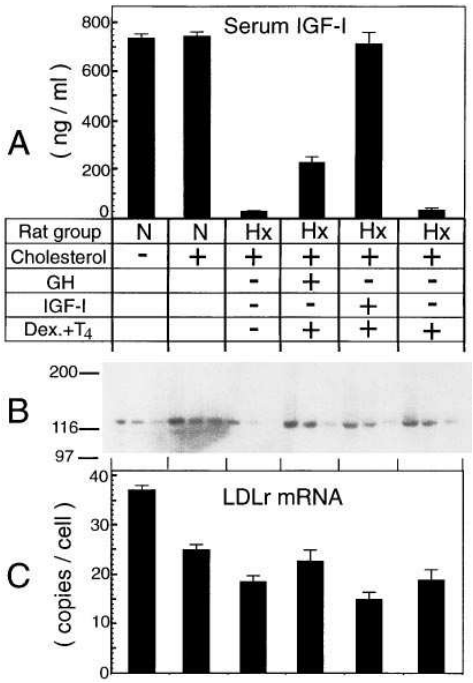

Figure 3. Different effects of GH and IGF-I in supporting the resistance of hepatic LDL receptors to suppression after dietary cholesterol. Normal (*N*) and Hx rats received 2% dietary cholesterol. Three groups of cholesterol-fed Hx rats received hormonal substitution by continuous subcutaneous infusion of the indicated hormones. After 6 d of treatment, rats were killed at 10:00 a.m. (*A*) Plasma IGF-I levels determined by radioimmunoassay on each individual. (*B*) LDL receptor determination by ligand blot. Hepatic membranes derived from pooled liver samples were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and subjected to ligand blotting using rabbit ^{125}I–β-VLDL. Three lanes were run for each group of animals (200, 100, and 50 μg of membrane protein, respectively). The picture was generated using a Bio-imaging analyzer. Data of the quantitated blot are shown in Fig. 5. (*C*) LDL receptor mRNA levels were quantitated in each individual by solution hybridization using hepatic total RNA as described. Bars represent SEM.

LDL receptor–binding activity, and LDL receptor mRNA levels increased by 25%. Animals substituted with Dex + T_4 had the same LDL receptor mRNA levels (19±1 copies per cell) as nonsubstituted Hx animals receiving cholesterol (19±0.5 copies per cell), although the LDL receptor expression was somewhat elevated. When these rats were compared with those receiving IGF-I + Dex + T_4, the latter group had lower receptor expression both at the protein and at the mRNA level (15±0.6 copies per cell). Thus, also in this model, GH substitution together with Dex + T_4 resulted in normalized LDL receptor expression and increased levels of LDL receptor mRNA. In contrast, IGF-I–treated animals showed, if anything, reduced LDL receptor mRNA receptor expression.

The results from the assay of liver total cholesterol in this experiment are shown in Table II. Treatment of normal rats with dietary cholesterol increased hepatic cholesterol sevenfold. In Hx rats receiving the same treatment, hepatic cholesterol levels were increased 11-fold. Substitution of cholesterol-fed Hx rats with GH + Dex + T_4 resulted in a dramatic (73%) reduction of hepatic cholesterol, and omission of only GH in a clear (twofold) increase. When cholesterol-fed Hx animals received IGF-I + Dex + T_4, a 47% reduction of the hepatic cholesterol level was obtained. Substitution of cholesterol-fed Hx rats with only Dex + T_4 resulted in a similar reduction of the hepatic cholesterol level (53%). In all situations, the major change in liver cholesterol was in the ester fraction (data not shown).

Plasma total cholesterol levels were slightly increased (+ 25%) in normal rats after dietary cholesterol (Table II). Cholesterol feeding of Hx rats resulted in a fivefold increase of plasma cholesterol. Substitution of cholesterol-fed Hx rats with GH + Dex + T_4 reduced plasma cholesterol levels by 35%, and substitution with IGF-I + Dex + T_4 gave a similar reduction of plasma cholesterol levels (30%).

Thus, despite their differing effects on hepatic LDL receptor expression, GH and IGF-I both reduced plasma total cholesterol to the same extent in cholesterol-fed Hx rats. However, when a detailed analysis of plasma lipoproteins after FPLC separation was made (Fig. 4), clear differences were observed. In line with previous experiments (24), the challenge of normal rats with dietary cholesterol only resulted in a limited increase of cholesterol within VLDL and IDL particles, whereas cholesterol in LDL and HDL particles was slightly reduced. When Hx animals received dietary cholesterol, a dramatic increase was observed (Fig. 4 *A*). Substitution of cholesterol-fed Hx animals with GH + Dex + T_4 resulted in a pronounced reduction of cholesterol in most lipoprotein fractions, whereas HDL cholesterol increased about threefold (Fig. 4 *B*). Omission of GH from the otherwise fully substituted animals resulted in a clear increase in cholesterol among IDL and LDL particles, whereas HDL cholesterol was reduced. Hx animals receiving IGF-I + Dex + T_4 had a pronounced reduction of cholesterol within VLDL and IDL particles. However, among LDL particles, little or no change was observed as compared with unsubstituted cholesterol-fed Hx animals. In all situations, the changes in lipoprotein cholesterol were parallel to the changes observed in apolipoprotein distribution as assessed by SDS-PAGE (data not shown).Thus, although plasma total cholesterol was reduced to the same extent in both groups, the plasma lipoprotein changes after GH substitution showed a completely different pattern as compared with that after substitution with IGF-I.

The different effects of GH and IGF-I on the plasma lipoprotein pattern may have several explanations. Previous studies have shown that GH is an important factor promoting the editing of apo B, and hence the hepatic production of apo B-48, in the rat (28). To study the relative importance of IGF-I in this regulation, we determined the editing of apo B in the above experiments (Fig. 5). In the cholesterol feeding experiment, the overall regulation of the apo B editing was strikingly similar to what was found for the LDL receptor regulation (Fig. 5 *B*). In agreement with a previous report on normal rats (40), cholesterol feeding of control rats resulted in slightly reduced apo B editing, from 61 to 47%. In cholesterol-fed Hx rats, the editing was reduced to 17%, and substitution with only Dex + T_4 was practically without effect, the editing of apo B being 23%. Further addition of IGF-I only marginally increased apo B editing, from 23 to 28%. However, after substitution with GH together with Dex + T_4, the apo B editing increased to 40%, a value close to that found for normal rats fed cholesterol. This pattern was confirmed in repeated experiments. Determination of apo B editing in estrogen-treated normal rats showed, again in agreement with previous reports (40, 41), that treatment with this hormone clearly reduced apo B editing, from 53 to 29% (Fig. 5 *A*). Estrogen-treated Hx rats had only 9% apo B editing. In this model, substitution with only Dex + T_4 was sufficient to fully normalize the editing of apo B (28%) compared with normal estrogen-treated animals. The additional substitution with IGF-I or GH increased the editing to 36 and 42%, respectively.

In addition, the two animal experiments mentioned above were performed with identical designs, using five animals in

Table II. Different Effects of GH and IGF-I on Cholesterol Metabolism, after Feeding of Rats with 2% Dietary Cholesterol as Described in Legend to Fig. 3

Group	N	N + Cholesterol	Hx + Cholesterol	Hx + Cholesterol	Hx + Cholesterol	Hx + Cholesterol
Hormonal substitution				Dex + T_4 + GH	Dex + T_4 + IGF-I	Dex + T_4
	A	*B*	*C*	*D*	*E*	*F*
Plasma cholesterol (mM)	2.10±0.04	2.62±0.05*	10.8±0.42‡	7.00±0.11‡§‖	7.55±0.20‡§‖	10.1±0.18‡
Plasma triglycerides (mM)	1.50±0.06	1.23±0.10	0.77±0.06	1.57±0.13§	0.82±0.05	1.48±0.17
Total hepatic cholesterol (mg/g)	3.93±0.03	30.2±1.44*	43.1±0.83‡	11.80±0.58‡§‖¶	22.7±0.34§	20.1±0.85‡§

Plasma total cholesterol and triglycerides and hepatic cholesterol were assayed in each individual as described in Methods. Values presented are mean± SEM. Abbreviations are as in Table I. Statistically significant difference ($P < 0.05$ or better) compared with *A, ‡B, §C, ‖F, ¶E.

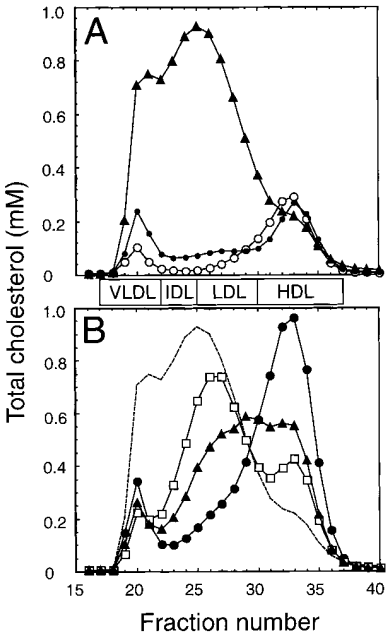

Figure 4. Plasma lipoprotein patterns of cholesterol-fed animals described in the legend to Fig. 3. FPLC separation was performed as described in the legend to Fig. 2. (*A*) Lipoprotein patterns of normal rats on regular chow (○), normal rats on 2% cholesterol chow (●), and Hx rats receiving 2% cholesterol (▲). (*B*) Lipoprotein patterns of cholesterol-fed (2%) Hx rats receiving hormonal substitution. Substitution with GH + Dex + T_4 (●), substitution with IGF-I + Dex + T_4 (□), and substitution with Dex + T_4 only (▲). For comparison, cholesterol-fed Hx rats not receiving hormonal substitution from *A* are included (- - - - - -).

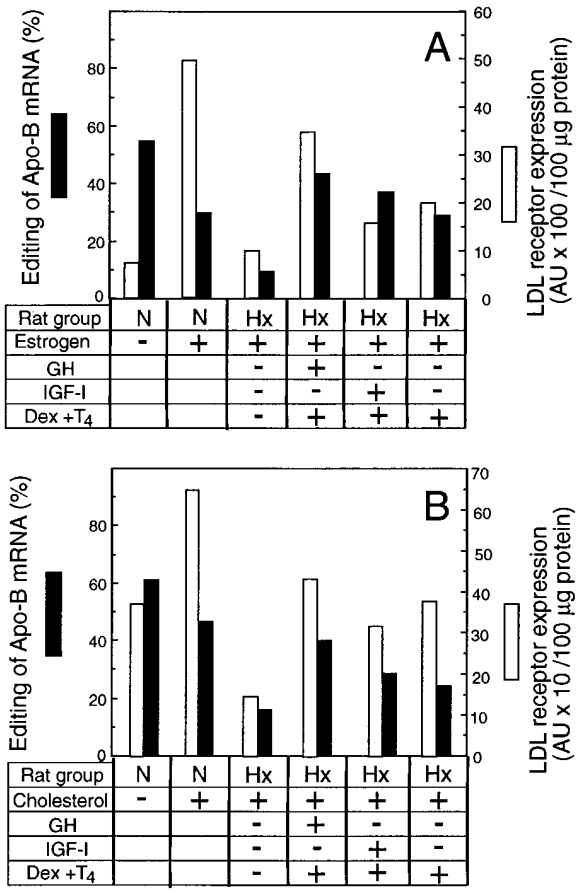

Figure 5. Editing of apo B mRNA in relation to expression of hepatic LDL receptors. *A* shows data from estrogen-treated animals, and *B* shows the data obtained from the cholesterol feeding experiment. For each group, apo B mRNA editing was obtained from analysis of pooled RNA from all individuals of each group. The LDL receptor expression was determined from the ligand blots in Figs. 1 and 3 using a Bio-imaging analyzer. *N*, normal rats.

each group, but with lower substitution doses (IGF-I, 200 µg injected twice daily, equivalent to 2 mg/kg per d; GH, 5 mg/h, equivalent to 0.6 mg/kg per d). In those experiments, the plasma levels of IGF-I in IGF-I–substituted Hx animals never reached normal physiologic concentrations; they were ~ 60% of those found in normal animals. Nevertheless, the results of these experiments were similar to those presented above (not shown).

Discussion

The present study clearly demonstrates that IGF-I and GH have different effects on the expression of hepatic LDL receptors in two, principally different, rat models in which the importance of GH for the stimulation of hepatic LDL receptors was previously established (20, 24). Furthermore, the responses to IGF-I and GH in plasma lipoprotein pattern were clearly different and in consonance with the observed effects on hepatic LDL receptors. Preliminary experiments suggested that a similar difference may exist between the two hormones as regards the effect on apo B mRNA editing, which might contribute further to the difference in plasma lipoprotein response.

In the present studies, recombinant human IGF-I and recombinant human GH were used. Both these compounds have previously been shown to have indistinguishable effects in rats compared with those obtained after administration of homologous compounds (42). In both animal models, the plasma levels of IGF-I after substitution with this hormone were the same as those observed in intact rats under similar dietary or pharmacological conditions, whereas the plasma levels of IGF-I were generally somewhat lower during substitution with GH. This may partly be due to the fact that higher IGF-I levels are obtained when GH is administered in a pulsative pattern (43). At any rate, the fact that GH administration, in spite of resulting in lower plasma IGF-I levels, specifically stimulated hepatic LDL receptors clearly supports the concept that IGF-I does not mediate these effects. This indicates that, in analogy with their effects on glucose and free fatty acid homeostasis, IGF-I and GH have different effects on hepatic LDL receptor expression.

In agreement with our observed changes in hepatic LDL receptor expression, there were consonant changes in plasma lipoproteins. Thus, VLDL, IDL, and LDL cholesterol levels were clearly much more affected by GH than by IGF-I substitution. Furthermore, in the estrogen-treated animals, plasma triglycerides were clearly lower in the GH- than in the IGF-I–substituted animals (Table I). On the other hand, plasma triglycerides were higher after GH substitution to cholesterol-fed Hx rats (Table II). As reviewed elsewhere (25), GH influences several important regulatory steps of lipoprotein synthesis and degradation. Since the editing of apo B mRNA is reduced in

Hx animals and stimulated by GH (28), and since the editing is also reduced by pharmacological treatment with estrogen (40, 41) and by feeding a cholesterol-enriched diet (40), different effects on the apo B mRNA editing process may play an additional role in the explanation of the differences in plasma lipoprotein pattern. Our present results (Fig. 5) would be in agreement with such an interpretation. Other aspects of lipoprotein metabolism, such as the stimulation of hepatic triglyceride and VLDL secretion (26, 27) and hepatocyte apo E and apo B secretion (30), were not assessed in the present study. However, a recent study by Sjöberg et al. (43) demonstrated that IGF-I and GH may also have different influences on these functions. Thus, plasma triglyceride and apo B levels were increased in IGF-I–substituted Hx adult female rats, whereas no effect or a decrease (apo B) was seen in GH-substituted animals. In addition, the elevation of plasma apo E levels observed in animals infused continuously with GH was not observed in IGF-I–substituted rats (43). That study also showed that the proportion of apo B-48 and the amount of triglyceride secreted from isolated hepatocytes were not affected by IGF-I substitution. These findings contrast with previously reported changes during GH substitution (28, 30) and clearly support our preliminary findings of different effects of the two hormones on apo B mRNA editing.

Lipid lowering effects of IGF-I treatment have been reported in patients with non–insulin-dependent diabetes (44). In contrast, in a recent, randomized crossover study of patients with primary osteoporosis in whom GH treatment increased plasma triglycerides and decreased LDL cholesterol levels, no significant effects were seen during IGF-I therapy (45). This finding would indicate a lack of stimulation of hepatic LDL receptors in response to IGF-I treatment in humans, contrasting with the known stimulation of liver LDL receptors that has been demonstrated directly during GH treatment in gallstone patients (20). At higher doses of IGF-I, insulin receptor–mediated effects on lipoprotein metabolism may still occur, which could be an explanation for the positive effects on lipoprotein metabolism observed in patients with type II diabetes (44).

The clear lack of effect on hepatic LDL receptors in response to IGF-I in intact rats reported here contrasts with the previously reported stimulation of LDL receptor expression on cultured swine granulosa cells (33) and human monocyte–derived macrophages (34). Interestingly, we could demonstrate recently an increased LDL receptor activity, as well as enhanced LDL receptor mRNA levels, in cultured human hepatoma Hep G2 cells exposed to IGF-I (46). In this cell line, incubation with GH also increased LDL receptor activity and mRNA levels, and, in contrast to the effect of IGF-I, the GH-induced response could not be inhibited by an mAb against IGF-I. Thus, the results of these in vitro experiments further support our conclusion from the present in vivo studies that GH stimulates the hepatic LDL receptor by an IGF-I–independent mechanism of action. One possible explanation for the obvious discrepancy regarding direct IGF-I stimulation of LDL receptors between in vitro and in vivo systems may be related to the distribution of specific IGF-I receptors. It has been shown that IGF-I receptors are barely detectable in normal adult rat liver, whereas this receptor is readily demonstrated in differentiating rat liver as well as in cultured hepatocytes (47). The number of IGF-I receptors appears to be low in human liver, also (47). These observations strongly emphasize the necessity of performing in vivo studies to establish the physiological relevance of hormonal LDL receptor expression regulation.

In conclusion, our study establishes that pituitary GH is of specific importance both for the normal stimulation of hepatic LDL receptors by estrogen (20) and for the resistance of this receptor to dietary cholesterol (24). Future studies should now focus on a further elucidation on the mechanisms by which GH specifically stimulates hepatic LDL receptors. Since GH seems to have both permissive and direct stimulatory effects, its potential role in the treatment of hyperlipidemic states, and particularly as a possible agent for the prevention of the age-related increase in plasma LDL cholesterol (25, 48), should also be the subject of further study.

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