Growth Hormone Induces Low-Density Lipoprotein Clearance but not Bile Acid Synthesis in Humans

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Objective—Growth hormone (GH) induces hepatic low-density lipoprotein (LDL) receptors and lowers plasma cholesterol. We characterized the influence of GH treatment on plasma LDL clearance in normal humans and investigated the relative role of LDL receptor (LDLR) activity and stimulation of bile acid synthesis in subjects with different LDLR expression.

Methods and Results—Plasma clearance of autologous 125I-LDL was measured before and during 3 weeks of treatment with GH (0.1 IU/kg per day) in 9 healthy young males. Plasma LDL cholesterol was reduced by 13% and the fractional catabolic rate of LDL increased by 27%. More marked changes were seen in a patient with hypopituitarism substituted with GH (0.07 IU/kg per day) for 3 months. In a second study, GH dose-dependently reduced LDL cholesterol and increased Lp(a) levels in 3 groups of males: younger and elderly healthy subjects and heterozygous familial hypercholesterolemia (FH). No effect on bile acid synthesis measured by the plasma marker 7α-hydroxy-4-cholesten-3-one was observed. In an LDLR-deficient FH homozygote, LDL cholesterol was not affected by GH.

Conclusions—GH treatment reduces plasma LDL cholesterol by inducing LDL clearance. In humans, LDLR expression is a prerequisite for this effect, whereas it is not related to stimulation of bile acid synthesis. (Arterioscler Thromb Vasc Biol. 2004;24:349-356.)

Key Words: insulin-like growth factor-I ■ low density lipoprotein kinetics ■ familial hypercholesterolemia ■ lipoprotein (a)

Increased levels of plasma low-density lipoprotein (LDL) cholesterol are associated with an enhanced risk of atherosclerosis and coronary heart disease. LDL receptors (LDLRs) are of great importance in determining plasma cholesterol, as evident from the genetic disease, familial hypercholesterolemia (FH), in which LDLRs are reduced by ~50% (heterozygotes) or lost (homozygotes).1 Stimulation of LDLR activity in the liver is a major mechanism for reducing plasma LDL cholesterol levels in humans2,3 and may be achieved by inhibition of cholesterol biosynthesis or stimulation of bile acid production.4–6 Also, estrogen treatment markedly induces hepatic LDLRs.7 In all these situations, the plasma clearance of LDL is enhanced, whereas the LDL production may show a more variable response.8–11

We have previously reported that treatment of normal adults with growth hormone (GH) stimulates hepatic LDLRs to a magnitude similar to that seen during therapy with cholesterol synthesis inhibitors.12 GH treatment can reduce total and LDL cholesterol but increases lipoprotein(a) (Lp(a)) in normal13–15 and GH-deficient adults.16–18 In parallel to the reduced secretion of GH with aging in humans,14,19 plasma LDL clearance and bile acid synthesis are reduced,20,21 whereas plasma LDL cholesterol increases.19,22 In the rat, the age-dependent reduction of bile acid production and increase of plasma cholesterol can be reversed by GH treatment.23 Thus, it has been hypothesized that GH is important for the normal regulation of plasma cholesterol.19,24 This may occur through effects of GH on hepatic LDLRs and/or bile acid synthesis.23,25 Interestingly, the administration of GH can stimulate bile acid synthesis and reduce plasma cholesterol even in mice lacking LDLRs.26

A more detailed knowledge of the effects of GH on plasma LDL metabolism is therefore warranted. In the present study, we first show that GH stimulates the plasma clearance of LDL apoB, resulting in reduced plasma LDL cholesterol levels, whereas it has minor effects on LDL apoB synthesis. Further, we address the following questions. Is the basal expression of LDLRs important for the LDL-lowering effect of GH in humans? Is the effect of GH in humans related to stimulation of bile acid synthesis? Is the effect of GH on...
plasma Lp(a) related to the LDL-lowering effect? We investigated four groups of human subjects in whom the expression of LDLRs can be assumed to differ: (1) normal younger males; (2) normal elderly males; (3) middle-aged males heterozygous for FH; and (4) a homozygote FH child.

Materials and Methods

Subjects

In the LDL turnover study, 9 healthy male volunteers aged 24 to 41 years (mean±SEM: 32±2 years) participated. They were of normal weight, with BMIs ranging from 22.6 to 25.8 (24.2±0.43). Plasma cholesterol and triglycerides were below the 95th percentile of the Swedish population. There was no clinical or laboratory evidence of thyroid, kidney, heart, or liver disease, diabetes, or alcohol abuse; no medication was used. In addition, 1 male patient (age: 61 years, BMI: 30.6) with hypopituitarism after surgical removal of a non-secreting adenoma 2 years before the study was investigated. He received substitution therapy with testosterone, thyroid hormone, and corticosteroids.

For the dose–response study, 8 men with heterozygous FH (age: 45±5 years; BMI: 25.0±0.95) and 15 healthy male volunteers were studied. The diagnosis of FH was based on described criteria.27,28 Five patients had known mutations in the LDLR gene,29,30 two had FH–Helsinki, one had a deletion of exon 2-3, and 2 had point mutations (L93R, IVS 10-2 A>G). Lipid-lowering drugs were omitted 6 weeks before the study. The healthy males were recruited from 2 age groups: younger (28±1: range 23 to 35 years) and elderly (66±1: 62 to 70 years) subjects. Neither had clinical or laboratory evidence of diabetes, overweight, hypertension, or renal, thyroid, or hepatic disease; BMIs were 24.2±1.1 and 26.3±0.83, respectively.

In addition, one homozygous FH patient of Turkish origin was investigated. He was 6 years old and had characteristic xanthomas and plasma cholesterol of ~20 mmol/L without treatment. DNA analysis confirmed homozygosity for the LDLR gene mutation W556R, which has been reported to result in <5% of normal LDLR activity in cultured fibroblasts.30 His apoE genotype was apoE3/2. Although he had increased intima media thickness of the carotids, echocardiography and workload test results were normal.

Informed consent was obtained from all participants and from the parents of the child. The studies were approved by the Ethics Committee of the Karolinska Institute and by the Swedish Medical Products Agency.

Experimental Procedures

In the LDL turnover study, the subjects were studied twice: once in the basal state and once during GH treatment. They were followed-up as outpatients and given a standardized diet 5 days following and during each study period.10,20 Potassium iodide, 200 mg/day, was administered for 3 weeks as single subcutaneous injections of GH (Genotropin; Pharmacia, Sweden). 0.1 IU/kg per day was administered orally 5 days before and during the studies. Blood sampling was performed every 7 days in the morning after overnight fast, when blood pressure, body weight, biochemical safety tests, and side effects were monitored.

The homozygous FH child had an individual protocol. Initially, she was administered continued treatment with 0.6 mg/kg per day of atorvastatin (Lipitor; Pfizer, Sweden). She was first administered GH 0.1 IU/kg per day for 1 week in addition to atorvastatin; thereafter, both drugs were omitted for a 4-week washout period. GH (0.1 IU/kg per day) was administered for 1 week and then increased to 0.2 IU/kg per day. Colestipol 0.45 g/kg per day (Lestid; Pharmacia, Sweden) and atorvastatin 0.6 mg/kg per day were then added to GH treatment as described in Figure 5.

Assays

Plasma insulin was determined by radioimmunoassay (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) and serum IGF-1 by RIA.31 The concentrations of IGF-binding protein-1 were determined according to Pövoa et al32 (courtesy of Prof Kerstin Hall, Karolinska Institutet).

Plasma cholesterol and triglycerides were measured by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany), and lipoproteins were quantitated using a combination of ultracentrifugation and precipitation.33,34 For the analysis of Lp(a) and apoAI and apoB, immunoturbidimetric methods were used (DAKO A/S, Glostrup, Denmark). The level of LDL apoA protein (LDL apoB) was calculated by multiplying the LDL cholesterol level with the protein/cholesterol ratio in the isolated LDL.35 Plasma 7α-hydroxy-4-cholesten-3-one (C4) was analyzed as described,36 and unesterified lathosterol in plasma was quantitated by mass spectrometry.37

125I-LDL Preparation and Turnover

LDLs (density 1.02 to 1.063 g/mL) were prepared by sequential ultracentrifugation and labeled with 125I using the iodine monochloride method;37 30 to 60 μCi (1.1 to 2.2 MBq) of 125I-LDL apoB was injected exactly as described.38 Blood samples were collected at 10 minutes and at 2, 4, 6, 8, 10, 12, 24, and 36 hours after the injection. Thereafter, samples were collected daily for 14 days. Urine was collected in 24-hour portions during the whole study. The fractional catabolic rate (FCR) of 125I-LDL apoB was calculated from the slope of the plasma radioactivity decay curve using the two-compartment model of Matthews.38 In addition, FCR was also calculated independently from the urine/plasma (U/P) ratio.39 The absolute catabolic rate of LDL apoB, expressed as milligrams of apoprotein synthesized per day normalized for body weight, was estimated according to Langer et al39 as described.40

Statistical Analysis

Data are presented as mean±SEM or median (range). All parameters were analyzed for normal distribution before and after log transformation. The significance of differences between groups was tested by two-way repeated-measures ANOVA, followed by planned comparison of group means according to the method of Tukey. The correlations between IGF-I and Lp(a), LDL, and HDL cholesterol were calculated using Spearman’s rank-order correlation. Calculations were performed using Statistica 5.5 (StatSoft Scandinavia AB, Uppsala, Sweden).

Results

Turnover Study

When exogenous human recombinant GH was administered to healthy males, serum IGF-I levels increased ~3-fold after 1 week and remained elevated during the study period (Figure 1). There were no significant changes in blood glucose, serum insulin, or IGF binding protein-1 levels. The levels of IGF-I reached were clearly supranormal and comparable to those observed in patients with mild acromegaly.41

After 1 week of therapy, a clear and sustained reduction of plasma LDL cholesterol was observed, whereas there were no significant changes in total or HDL cholesterol or in plasma

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triglycerides (Figure 1). Also, body weight remained stable during the study, further indicating a steady state. The FCR of LDL apoB averaged 0.315 ± 0.015 pools per day in the basal state as calculated from analyses of plasma radioactivity decay curves (Figure 2). The calculated production rate of LDL apoB was 12.3 ± 1.2 mg/kg per day. Both these values are in good agreement with data obtained previously in healthy males of similar ages.20 Data from urine/protein analyses were in excellent agreement with those obtained from analyses of plasma data (not shown).

A clear increase in the elimination rate of LDL apoB was observed in response to GH treatment (0.399 ± 0.010 pools per day; \( P < 0.0023 \); Figure 2). Plasma LDL apoB levels were reduced by 14%, from 101 ± 7 to 87 ± 5 mg/dL (\( P = 0.037 \)). The estimated production rate tended to be slightly higher during therapy, but the difference was not statistically significant (13.3 ± 1.2 mg/kg per day; \( P = 0.14 \)).

It should be noted that the tracer used in the experiments consisted of LDL collected before GH treatment was initiated. Analyses of the LDL composition (mg%) before (cholesterol:triglyceride:phospholipid:protein = 40 ± 0.7:7 ± 0.7:29 ± 0.7:24 ± 0.7) and after 3 weeks of GH treatment (39 ± 0.7:10 ± 1.0:28 ± 0.7:23 ± 0.3) actually revealed an increase in the relative content of triglyceride (\( P = 0.002 \)). The calculations of LDL apoB flux before and during treatment should therefore not be regarded as fully comparable on a quantitative basis. However, the demonstration of an increased clearance of pre-treatment LDL particles in GH-treated subjects is strongly suggestive of an increased hepatic LDLR expression, because the problem of a possible difference in receptor binding affinity of particles isolated during treatment42–44 is avoided by the present approach.

In a separate experiment (Figure 3), one patient with hypopituitarism and GH deficiency was administered GH at substitution dosage (0.07 IU/kg per day). LDL apoB FCR in this patient increased 38% and the response was even more pronounced than in the normal subjects. Because of an increased production rate, plasma LDL apoB levels were virtually unchanged, whereas mean LDL cholesterol decreased from 4.5 to 3.8 mmol/L.

**Dose–Response Study**

All participants completed the study with excellent compliance and tolerated the treatment well. In response to GH treatment, plasma levels of IGF-I increased dose-dependently in all groups (Figure 4A). At baseline, younger subjects showed slightly higher IGF-I levels compared with levels of elderly subjects (\( P = 0.025 \)) and of FH patients (\( P = 0.05 \)). They also had a significantly more pronounced response after weeks 2 and 3. Also, plasma insulin and glucose levels increased dose-dependently in all groups (not shown), but no significant differences in responses were seen between groups.

The plasma total and LDL cholesterol levels were reduced by GH treatment in all 3 groups in a dose-dependent way (Table 1). The relative response was somewhat more brisk in
the younger subjects (Figure 4B), but there was no difference between the 3 groups after 3 weeks. HDL cholesterol levels were slightly but significantly reduced in all groups after 1 week, but there was no relationship to dose, and apoAI levels were unaltered. Plasma apolipoprotein B levels were significantly reduced in all groups after 3 weeks of treatment. FH patients had higher basal triglyceride levels (P=0.019) as compared with levels of healthy subjects. Plasma triglycerides increased dose-dependently in all groups. Lp(a) levels increased significantly in all groups in a dose-dependent manner. Although the IGF-I response to GH treatment was stronger in the younger subjects (Figure 4A), no correlations were observed between IGF-I levels and changes in LDL or HDL levels. In the younger subjects, no correlations were observed between IGF-I levels and changes in LDL or HDL levels. However, no significant differences in the relative responses between the groups (Figure 4C).

Although the IGF-I response to GH treatment was stronger in the younger subjects (Figure 4A), no correlations were observed between IGF-I levels and changes in LDL or HDL cholesterol or Lp(a) concentrations (r<0.10 in all cases).

The plasma level of the bile acid intermediate, 7α-hydroxy-4-cholesten-3-one (C4), reflects the activity of the rate-limiting enzyme in bile acid production, cholesterol 7α-hydroxylase. As seen in Table 1, GH treatment did not influence C4 levels in any group. The plasma ratio of unesterified lathosterol to cholesterol is a marker of hepatic and total body cholesterol synthesis. As displayed in Table 1, this marker was not influenced by GH treatment in any of the groups.

We finally investigated the effects of stepwise addition of GH, colestipol, and atorvastatin treatment in the girl with homozygous FH (Figure 5). Overall, there were no major plasma lipoprotein changes. LDL cholesterol was reduced by 9% when all drugs were combined. Plasma triglycerides increased in response to GH, and this was normalized on combination with atorvastatin.

**Discussion**

GH exerts many important effects in the regulation of lipid metabolism. In general, GH increases the flux of energy in the lipid transport system by stimulating lipolysis in adipose tissue, fatty acid assimilation and triglyceride production in the liver, very-low-density lipoprotein secretion and catabolism, LDL clearance, and cholesterol excretion. Some of these effects are only observed in GH deficiency where they can be normalized by GH substitution, whereas others also occur in response to supraphysiological GH doses. The present study allows some important conclusions regarding the effects of GH on lipoprotein metabolism in humans.

First, administration of exogenous GH to healthy subjects resulted in a clear increase in the catabolism of LDL particles. We have reported that GH treatment for 5 days increases LDLR as measured directly in the liver of patients undergoing cholecystectomy and that GH induces LDLR activity in cultured human hepatoma cells. In the present experiment, the whole-body capacity for LDL clearance was examined through the LDL turnover technique, which reflects the total LDLR activity. The present data thus confirm and extend our earlier in vitro findings in human liver tissue. The FCR of LDL was increased by 27% in the normal subjects during GH treatment. This increase in FCR is of the same magnitude as the response seen with cholestyramine feeding at a dosage of 24 grams daily. However, the lowering of LDL cholesterol by GH was less than that observed in response to cholestyramine, probably because the production rate is somewhat increased with GH treatment. Stimulation of very-low-density lipoprotein production has been observed in response to GH substitution in adult GH-deficient patients using stable isotope kinetics. In our patient with pituitary insufficiency, LDL apoB FCR increased by 38% on GH substitution, despite the fact that he was administered a lower dose of GH than were the healthy subjects. In this single subject, LDL apoB synthesis was increased by GH, indicating that the flux of lipoproteins through plasma may be stimulated. Thus, a stimulation of LDL clearance by GH could be demonstrated, supporting the concept that GH is an important hormone in the complex physiological control of plasma LDL flux in humans.

Second, GH treatment resulted in a dose-related lowering of LDL cholesterol in younger and elderly normal male subjects. Although the response to the lowest dose was somewhat less pronounced in the elderly, this probably reflects a lower sensitivity to GH, as judged from the IGF-I response. The increase of LDL clearance that we demonstrate in young males can thus probably also be elicited in elderly males when the initial LDL clearance is reduced and when a relative deficiency of, or insensitivity to, GH is present. However, in both groups the LDL cholesterol lowering was relatively moderate (=15% at the highest dose). The LDLR stimulatory effect seen with GH treatment may have several explanations. Hypothetically, this effect could be mediated by...
IGF-I, a somatomedin that increases in plasma after GH secretion. In the present study, we were able to demonstrate a 3-fold increase of IGF-I in response to the GH treatment, but there was no correlation between IGF-I levels and lipoprotein changes. Recent studies in humans\(^2\) and in rats\(^3\) have demonstrated that IGF-I treatment does not result in the same effects as GH treatment on plasma lipoprotein levels or on hepatic LDLR expression in vivo, which strongly indicates that the effect of GH treatment on LDLR and LDL catabolism is direct. The molecular mechanisms for this effect remain to be explored.

Third, the effects of GH on LDL cholesterol in heterozygous FH were similar to those seen in younger and elderly healthy subjects. Reductions in LDL cholesterol have also been observed in FH heterozygotes treated with 0.05 IU/kg per day for 12 weeks.\(^4\) Although probably of limited therapeutic value in heterozygous FH, it is evident that the capacity for LDL-lowering by GH was not decreased, despite a reduced initial expression of LDLR. However, our data from the receptor-deficient FH homozygote clearly indicate that the capability to express LDLR is a requisite for LDL-lowering effects of GH. Although only 1 patient was studied, this finding is in clear contrast to observations in LDLR-deficient (knockout) mice, in which GH treatment reduced plasma LDL cholesterol levels.\(^5\) Thus, there is a clear species difference in the lipid-lowering effect of GH. The explanation to this is unclear, but it may be mentioned that in rodents GH stimulates the hepatic editing of apoB, resulting in a faster clearance of hepatic lipoproteins from the circulation.\(^5\) This response should not occur in humans.\(^6\)
Also, the different effect of GH in stimulation of bile acid synthesis may be of importance for this species difference. Fourth, the administration of GH to normal human subjects does not increase bile acid synthesis, which is in agreement with previous results in which bile acid production was studied using isotope kinetics in young normal male subjects before and during treatment with GH. In the rat, bile acid synthesis is dependent on the presence of GH, and stimulation of cholesterol breakdown to bile acids can be achieved by pharmacological doses of GH. Thus, there is a difference between rodents and humans regarding the regulation of bile acid synthesis by GH. The metabolic basis behind this is unclear, but it is of interest to note that species differences have recently been described in the molecular regulation of bile acid synthesis. Furthermore, there appear to be species differences in the plasma lipoprotein response to stimulation of bile acid synthesis in LDLR deficiency. Thus, stimulation of cholesterol 7α-hydroxylase by gene transfer, or interruption of bile acid intestinal uptake lowers LDL cholesterol in LDLR-deficient knockout mice, whereas complete biliary diversion in homozygous FH does not lower plasma LDL.

Fifth, the concentration of plasma Lp(a) increased dose-dependently in all groups. Also in GH deficiency, Lp(a) levels are increased by GH substitution, whereas they are reduced after therapy of acromegaly. It has been shown that IGF-I does not increase Lp(a). Instead, its administration reduces Lp(a). Therefore, the present results strongly support the concept that GH directly stimulates the synthesis and secretion of apo(a), a mechanism demonstrated in mice transgenic for human apo(a). Although the clinical relevance of the GH-induced Lp(a) increase is not known, this consistent effect together with increased plasma levels of glucose, triglycerides, and reduced plasma HDL cholesterol should call for some caution in situations in which GH treatment is considered.

Acknowledgments
The skilful technical assistance of Lilian Larsson, Danuta Cosliani, and Sabine Sillö-Barin, and the manuscript preparation of Lena Emstom are gratefully acknowledged. The studies were supported by grants from the Swedish Medical Research Council (03X-7137), the Swedish Heart–Lung Foundation, the Loo and Hans Osterman and the Thuring Foundations, the Foundation of Old Female Servants, and the Karolinska Institute. Human growth hormone was kindly provided by Pharmacia, Stockholm, Sweden.

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Arterioscler Thromb Vasc Biol. 2004;24:349-356; originally published online December 4, 2003;
doi: 10.1161/01.ATV.0000110657.67317.90
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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