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Loss of resistance to dietary cholesterol in the rat after hypophysectomy: Importance of the presence of growth hormone for hepatic low density lipoprotein-receptor expression

(dexamethasone/thyroid hormone/metabolic regulation/atherosclerosis)

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ABSTRACT **This investigation was undertaken to determine the role of pituitary function and, in particular, the possible influence of growth hormone (GH) on hepatic low density lipoprotein (LDL)-receptor expression in response to dietary cholesterol. Feeding normal rats with 2% cholesterol for 5 or 6 days did not alter LDL-receptor numbers, LDL-receptor mRNA levels, or plasma cholesterol, although hepatic cholesterol increased 5-fold. When hypophysectomized rats received the same diet, the LDL-receptor number and its mRNA levels were reduced by 75%, plasma cholesterol increased 6-fold, and hepatic cholesterol increased 12-fold. Stepwise hormonal substitution of cholesterol-fed, hypophysectomized rats revealed that substitution with GH was important to restore hepatic LDL-receptor number and mRNA levels. The presence of GH was also important to reduce the hypercholesterolemia in cholesterol-fed hypophysectomized rats. We conclude that the presence of GH is important for hepatic LDL-receptor expression, both at the protein and the mRNA level. The resistance to suppression of rat hepatic LDL receptors by dietary cholesterol depends, at least in part, on the presence of GH.**

An increased concentration of plasma cholesterol and, in particular, of low density lipoprotein (LDL) cholesterol is associated with an enhanced risk of atherosclerosis and coronary heart disease (1, 2). Therapeutic reduction of total and LDL cholesterol can retard the development of coronary atherosclerosis (3–5). Regulation of hepatic LDL receptors is a major mechanism by which dietary and hormonal agents influence plasma cholesterol levels (6, 7). By controlling LDL catabolism, the number of hepatic LDL receptors directly influences the plasma LDL-cholesterol concentration. The number of LDL receptors depends on cholesterol availability, both in the liver and in extrahepatic tissues (6–8).

Upon ingestion of a cholesterol-rich diet, the hepatic LDL receptors are usually suppressed, contributing to a subsequent elevation of plasma LDL (9–11). There is a large interspecies variation as regards the extent of receptor suppression. Thus, rabbits and hamsters respond with a pronounced suppression of hepatic LDL receptors in response to cholesterol feeding, a marked hypercholesterolemia, and subsequent atherosclerosis (9, 12, 13). Species such as the rat and the mouse display little or no suppression of hepatic LDL receptors after dietary cholesterol (14, 15), and highly artificial diets are required to induce hypercholesterolemia and atherosclerosis in these animals (16–18). The mechanism for this resistance of hepatic LDL receptors to dietary cholesterol is unknown.

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We previously demonstrated that growth hormone (GH) is important for hepatic LDL-receptor induction after estrogen treatment of rats and that treatment of humans with GH could induce hepatic LDL receptors and lower plasma cholesterol (19). Furthermore, recent observations have indicated that GH-deficient human adults—frequently displaying elevated plasma cholesterols (20–22)—seem to exhibit premature atherosclerosis (23) and increased cardiovascular mortality (24, 25). Because, compared with humans, GH secretion is high in the rat (26–28), a fundamental question emerges: Has GH a role in the resistance to dietary cholesterol in the rat? To answer this question, we studied hepatic LDL-receptor expression and plasma lipoproteins in normal and hypophysectomized (Hx) rats fed a diet enriched with cholesterol.

In contrast to normal rats, Hx rats were very sensitive to dietary cholesterol, displaying a strong suppression of hepatic LDL receptors concomitant with a drastic increase of plasma cholesterol. Hormonal-replacement experiments showed that the presence of GH was important to normalize the expression of hepatic LDL receptors in these animals.

MATERIALS AND METHODS

Materials. Cholesterol was from Sigma (no. C-8503). The cholesterol diets (0.15–2%) were made by mixing ground rat chow with hot Mazola corn oil, 9:1 (CPC Foods AB, Kristianstad, Sweden), into which cholesterol had been dissolved. All other materials were from described sources (19).

Animals and Experimental Procedure. Altogether, 91 male Sprague–Dawley rats were used. They had free access to water and chow; the light cycle was from 6 a.m. to 6 p.m. Hypophysectomy was performed on 200-g rats by a parapharyngeal approach. Body weight was controlled weekly to verify failure to gain weight. Rats received the cholesterol diet 3 weeks after hypophysectomy.

Rats received the cholesterol diet for 5 or 6 days. Infusion of hormones (1 μl/hr) was started by s.c. implantation, under ether anesthesia, of minipumps 42 hr before cholesterol feeding. Human GH (in separate minipumps) was infused at a rate of 5 μg/hr, dexamethasone (Dex) was infused at 0.8 μg/hr, and L-thyroxin (T_4) was infused at 0.35 μg/hr. After 5 or 6 days of cholesterol feeding, rats were sacrificed between 11 a.m. and 2 p.m. Animals were anesthesized with ether, bled by heart puncture, and thereafter killed by cervical dislocation. Plasma and liver samples were obtained, and the latter were frozen in liquid nitrogen.

Abbreviations: apo, apolipoprotein; β-VLDL, β-migrating very low density lipoprotein(s); Dex, dexamethasone; FPLC, fast protein liquid chromatography; GH, growth hormone; HDL, high density lipoprotein(s); Hx, hypophysectomized; IDL, intermediate density lipoprotein(s); LDL, low density lipoprotein(s); T_4, L-thyroxin; VLDL, very low density lipoprotein(s).

Ligand Blot Assay of LDL Receptors. Hepatic membranes were prepared from pooled liver samples of each group and separated on nonreduced SDS/PAGE (6%), transferred to nitrocellulose filters, and incubated with ^{125}I-labeled rabbit β-migrating very low density lipoprotein (β-VLDL), as described (19). Filters were exposed on Kodak XAR film for the indicated times (19).

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

Lipoprotein Characterization. Size-fractionation of lipoproteins by fast protein liquid chromatography (FPLC) was done as described (15, 29, 30). In brief, 3.5 ml of pooled plasma was concentrated by ultracentrifugation, and 2 ml of this (corresponding to 2 ml of plasma) was injected onto the column. The sites of elution of very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), LDL, and high density lipoproteins (HDL) were established from determination by SDS/PAGE (4–20%) of apolipoproteins (apos) within each fraction (29).

Cholesterol Assay. Hepatic cholesterol was extracted from liver samples as described (15). Cholesterol in plasma, FPLC fractions, and liver extracts was assayed with a Nycotest kit (Nycomed, Oslo), using a 5.2 mM cholesterol standard (Merck; no. 14164). Individual samples were run in duplicate, except for plasma cholesterol presented in Fig. 1*A*, where data from duplicate assays of pooled samples are shown.

Statistical Methods. Data are means and SEMs. Significances of differences were tested with the two-tailed Student's *t* test (31). In the experiment of Fig. 4, the effects of GH only, Dex plus T_4 only, and the presence of interaction when GH was combined with Dex plus T_4 were evaluated by factor analysis, as described for a 2^2 factorial experiment (31). Correlations were tested by calculating the correlation coefficient, *r* (31).

RESULTS

To study the role of the pituitary for the response to dietary cholesterol, we compared normal and Hx rats after feeding with normal and cholesterol diets. After 6 days on a diet with 2% cholesterol, the total plasma cholesterol was unaltered in normal rats, although hepatic cholesterol increased 5-fold ($P < 0.005$, Fig. 1*A*). Hypophysectomy alone did not clearly alter total plasma or hepatic cholesterol levels, but when Hx animals received dietary cholesterol, the plasma levels increased 6-fold while hepatic cholesterol increased 12-fold ($P < 0.001$). As shown in Fig. 1*B*, feeding normal rats with cholesterol did not reduce hepatic LDL-receptor expression; if anything, a slight stimulation was seen. Hx animals displayed reduced (≈50%) hepatic LDL-receptor binding. When Hx rats were fed cholesterol, a pronounced reduction of hepatic LDL-receptor binding was observed. Analysis of the mRNA levels for the LDL receptor showed similar results (Fig. 1*C*). Thus, no alteration occurred upon feeding normal rats the cholesterol diet. Despite reduced LDL-receptor binding, Hx rats had slightly increased LDL-receptor mRNA levels, but when Hx rats were fed cholesterol, the receptor-transcript levels were reduced to one-fourth of those observed in Hx rats on a regular diet ($P < 0.001$).

To characterize plasma lipoprotein changes, lipoproteins were separated by FPLC after ultracentrifugation of plasma from each group. In spite of having similar total plasma cholesterol levels as normal rats, Hx rats showed a different pattern as compared with normal rats (Fig. 2*A*). Thus, a separate peak, within the size of LDL particles (fractions 26–30), was present in plasma from Hx rats, while the cholesterol within HDL particles (fractions 31–38) tended to

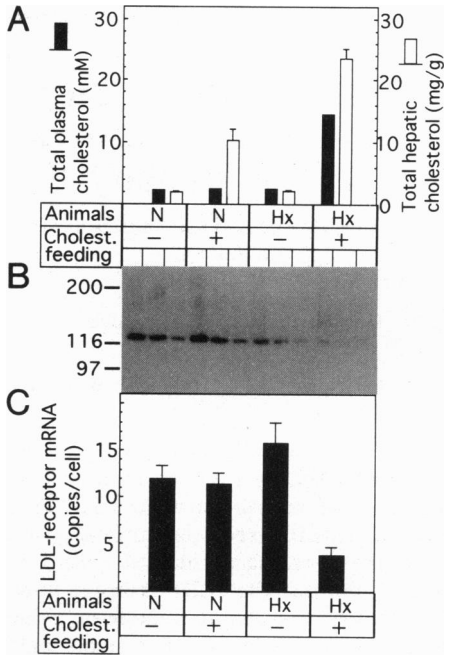

FIG. 1. Role of the pituitary for effects of dietary cholesterol on total plasma and hepatic cholesterol levels (*A*), expression of hepatic LDL-receptor binding (*B*), and hepatic LDL-receptor mRNA (*C*). Normal (N) and Hx rats (Hx) received rat chow supplemented with 2% cholesterol for 6 days before killing. Each group was comprised of five animals. Bars indicate SEM. *B* shows a ligand blot, using ^{125}I-labeled β-VLDL on SDS/PAGE-separated hepatic membranes from pooled liver samples of the indicated animal groups. For each group, lanes were loaded with 200, 150, and 100 μg of protein, respectively. The blot was exposed for 2 hr.

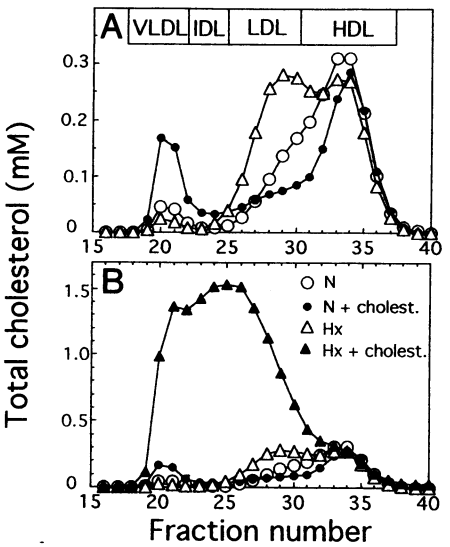

FIG. 2. Characterization of plasma lipoprotein patterns in normal (N) and Hx rats with and without challenge with 2% dietary cholesterol (cholest.). Blood plasma samples from the groups of animals described in the legend to Fig. 1 were pooled (3.5 ml) and concentrated by ultracentrifugation. Two milliliters of the concentrated lipoproteins (corresponding to 2 ml of plasma) was thereafter separated by FPLC, as described. Symbols for the respective animal groups are indicated in *B*. (*A*) Lipoprotein patterns of normal and Hx rats and pattern of cholesterol-fed normal rats. (*B*) Lipoprotein pattern of cholesterol-fed Hx rats is related to those of all other animal groups.

decrease. Further analysis of the fractions by SDS/PAGE revealed an increase in apo B-100 within the LDL fraction of the Hx rats (data not shown). When normal rats received the cholesterol diet, the cholesterol in plasma increased predominantly within VLDL (fractions 18–22) and IDL (fractions 23–25), while cholesterol in large HDL was reduced. Analysis of plasma from cholesterol-fed Hx rats showed a profound increase of cholesterol within the VLDL, IDL, and LDL fractions (Fig. 2*B*). This increase was associated with an increase of apo B-100 in the VLDL, IDL, and LDL fractions (data not shown). When groups of Hx rats received increased concentrations of dietary cholesterol for 6 days, a clear dose-dependent response was present even upon feeding Hx animals 0.15% cholesterol (Fig. 3*A*). Plasma cholesterol increased predominantly within LDL and IDL particles (Fig. 3*B*).

Thus, hypophysectomy turned the rats into animals with elevated LDL-cholesterol levels, which upon challenge with dietary cholesterol responded with a reduced hepatic LDL-receptor expression and a clear increase of cholesterol within LDL, IDL, and VLDL particles. This result suggests that the pituitary has a crucial role to maintain the rat resistant to dietary cholesterol. To determine the possible role of GH in this resistance, Hx rats were fed the 2% cholesterol diet, and groups of animals received hormonal substitutes of GH alone, GH plus Dex, GH plus Dex plus T_4, and Dex plus T_4 (Fig. 4). In addition, two groups of normal rats again received normal and cholesterol-enriched chow, respectively. After 5 days on the cholesterol diet, plasma and liver samples were collected. In this experiment, normal rats fed cholesterol had a slightly increased total plasma cholesterol, while hepatic cholesterol again increased 5-fold (Fig. 4*A*). Total plasma and hepatic cholesterols were again greatly increased in Hx rats on the cholesterol diet. When cholesterol-fed Hx rats received GH alone, total plasma cholesterol was reduced, whereas additional substitution with Dex, or Dex plus T_4 did not reduce the total plasma cholesterol levels further. Hepatic cholesterol was much reduced when Dex was included in the substitution. Omission of GH from the "fully" substituted animals resulted in elevated total plasma cholesterol levels and a 2-fold increase of hepatic cholesterol.

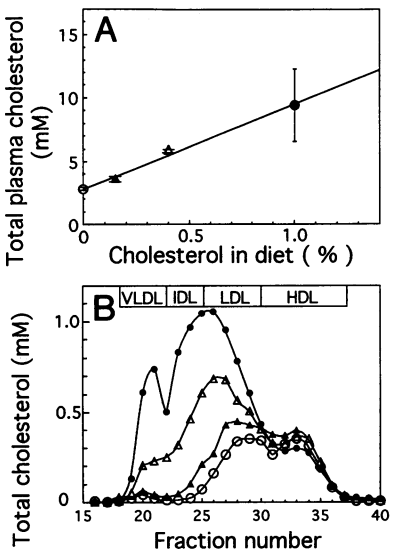

FIG. 3. Sensitivity to dietary cholesterol in Hx rats. Hx rats received the indicated concentrations of dietary cholesterol for 6 days before sacrifice. (*A*) Relation between the dose of cholesterol and total plasma cholesterol. Each group was comprised of four animals, except the controls (n = 3). Bars indicate SEM. (*B*) Cholesterol concentrations in FPLC-separated pools of concentrated lipoproteins. Symbols used in *B* are derived from those of *A*.

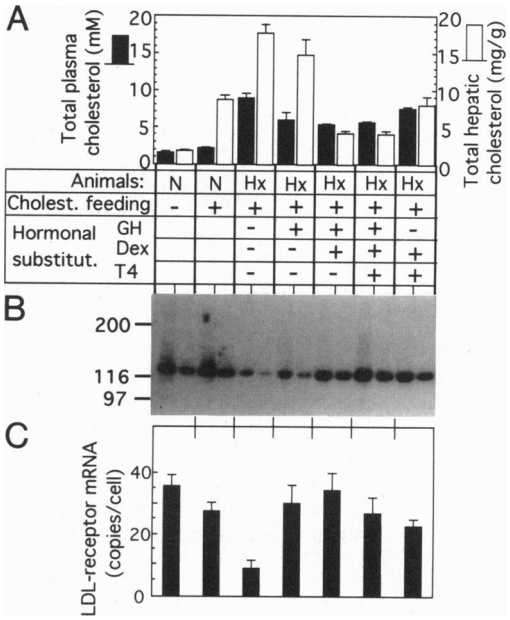

FIG. 4. Effects of hormonal substitution of cholesterol-fed Hx rats on plasma and hepatic cholesterol (*A*), hepatic LDL-receptor binding (*B*), and hepatic LDL-receptor mRNA (*C*). On day 0, Hx rats were implanted with s.c. osmotic minipumps delivering the indicated hormones as described. Nonsubstituted rats were sham-operated. On day 2, cholesterol feeding (2%) was initiated in the indicated groups. On day 7, after 5 days of cholesterol feeding, all rats were killed, and tissues were collected as described. Each group was comprised of four animals. Bars indicate SEM. (*B*) Ligand blot, using ^{125}I-labeled β-VLDL on SDS/PAGE-separated hepatic membranes from pooled liver samples of the indicated animal groups. For each group, lanes were loaded with 200 and 100 μg of protein, respectively. The blot was exposed for 3 hr. Factor test for significance (effect of GH only, Dex plus T_4 only, or the presence of interaction when GH was combined with Dex plus T_4) showed a significant effect of GH on plasma cholesterol ($P < 0.005$), hepatic cholesterol ($P < 0.025$), and LDL-receptor mRNA ($P < 0.025$). The effect of Dex plus T_4 was only significant for hepatic cholesterol ($P < 0.025$). No significant interaction was present when GH was combined with Dex plus T_4.

Fig. 4*B* shows that the receptor binding was not reduced among normal rats fed 2% cholesterol as compared with controls; if anything, there was again higher binding. When Hx rats received the cholesterol diet, the LDL-receptor expression was again suppressed. Addition of GH resulted in an increased expression of hepatic LDL receptors. A further increase was obtained by the combined infusions of Dex and GH. Animals on full hormonal substitution showed similar LDL-receptor expression as normal rats fed cholesterol. Omission of GH alone reduced the expression of hepatic LDL receptors. Analysis of LDL-receptor mRNA levels showed that GH was of major importance for normalizing receptor-transcript levels (Fig. 4*C*). However, when Dex was given with GH, the binding activity in the ligand blot clearly increased, despite relatively unchanged mRNA levels (Fig. 4 *B* and *C*). Factor analysis of this experiment showed that the effects of GH treatment were statistically significant (legend to Fig. 4).

When the LDL-receptor mRNA levels of the groups of Hx animals were plotted against the logarithmically transformed total plasma cholesterol in the respective groups, a strong negative correlation was obtained (Fig. 5). The importance of the presence of GH for the LDL-receptor transcript levels is particularly evident in this graph.

We finally characterized in detail the plasma lipoprotein changes in all animal groups of this experiment (Fig. 6). When normal rats received cholesterol in the diet, it was again

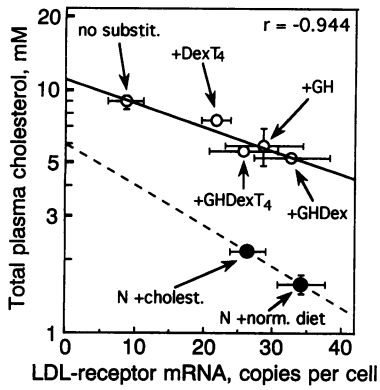

FIG. 5. Relation between hepatic LDL-receptor mRNA levels and total plasma cholesterol for the groups of rats described in the legend to Fig. 4. Bars show SEM. Coefficient of correlation (r) for Hx rats (○) is −0.944; solid line represents regression line for these animals. ●, Normal rats (N); the broken line connects mean data points. substit., hormonal substitution; norm, normal; cholest., cholesterol.

found that cholesterol increased within VLDL and IDL particles, while cholesterol among HDL particles decreased (compare Figs. 6*A* and 2*A*). Cholesterol-fed Hx rats again showed a drastic increase of cholesterol among LDL and IDL particles. When cholesterol-fed Hx rats were infused with GH, a clear decrease in cholesterol, particularly among IDL and LDL particles, occurred (Fig. 6*B*). Additional infusion with Dex caused a further decrease of cholesterol among VLDL and IDL particles. HDL cholesterol increased severalfold on this treatment, whereas LDL cholesterol was not clearly altered (Fig. 6*B*). Full hormonal substitution by the addition of T_4 did not further reduce cholesterol among plasma lipoproteins (Fig. 6*C*). However, when GH was omitted from the otherwise fully hormonally substituted animals, a clear increase of cholesterol within IDL and LDL particles occurred, while cholesterol in HDL was reduced (Fig. 6*C*). Parallel changes in apo B-100 were observed as determined from SDS/PAGE: furthermore, apo A-I increased when Dex was included in the substitution (data not shown).

The entire experiment presented in Figs. 4–6 was repeated, except that rats were fed with 0.5% dietary cholesterol; results were the same as presented above (data not shown). Thus, all results presented were highly reproducible.

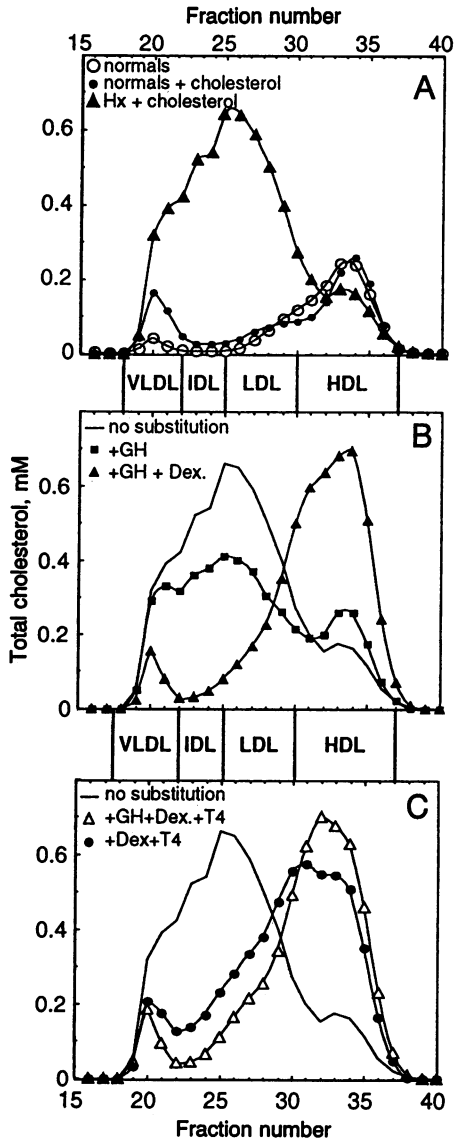

FIG. 6. Characterization of plasma lipoprotein pattern in normal and Hx rats upon dietary cholesterol load and after hormonal substitution of cholesterol-fed Hx rats. Blood plasma samples from animals of the experiment described in the legend to Fig. 4 were pooled (3.5 ml), and concentrated lipoproteins (corresponding to 2 ml of plasma) were thereafter separated by FPLC, as described.

DISCUSSION

Several important conclusions may be drawn from our investigation.

(*i*) The pituitary is essential for maintaining the characteristic plasma lipoprotein pattern in the rat because hypophysectomy changed the lipoprotein spectrum from a predominant HDL pattern to one with a distinct LDL peak. (*ii*) The resistance to down-regulation of hepatic LDL receptors seems important for the inability of this animal to develop hypercholesterolemia after cholesterol feeding. (*iii*) This resistance is heavily dependent on normal pituitary function because hypophysectomy resulted in a down-regulation of hepatic LDL-receptor expression, both at the protein and at the mRNA level. (*iv*) The presence of GH is important for maintaining the hepatic LDL receptor resistant to suppression by dietary cholesterol in the rat. Thus, the presence of GH seems to be important for hepatic LDL-receptor expression, not only under extreme stimulatory conditions such as high-dose estrogen treatment (19) but also under more physiological conditions. This result suggests that GH may have an important general role in the overall regulation of hepatic LDL-receptor expression.

The underlying molecular mechanism(s) explaining how the presence of GH exhibits its stimulatory action on hepatic LDL receptors is unknown. The increases of both receptor mRNA and receptor protein indicate that gene transcription may be involved. An important question is whether GH or its messengers act on the LDL-receptor gene or whether the effects are indirect and due to cellular steroid (cholesterol) depletion; the latter might be secondary to GH-induced effects on hepatic lipoprotein synthesis or biliary cholesterol and bile acid excretion. The role of Dex or T_4 was not addressed here. The fact that the addition of Dex to the GH substitution resulted in a further stimulation of receptor-binding activity, despite a minor change in mRNA levels (Fig. 4 *B* and *C*), may indicate that this hormone is important for translational efficiency. The relatively small effects of the T_4 addition to the substitution may be because some effects of thyroid hormones on lipoprotein metabolism are GH-mediated (32).

The plasma lipoprotein changes in Hx rats receiving cholesterol could not be normalized with GH or with full substitution. The particular magnitude of substitution used in the present study, by using three different agents, may contribute to the reason why plasma lipids could not be fully normalized because an optimal substitution regimen is complex and, hence, difficult to achieve. The addition of Dex resulted in elevated HDL and apo A-I levels compared with normal rats (Fig. 6*B*), which may be related to the fact that Dex increases plasma apo A-I levels more potently than does hydrocortisone (33). Furthermore, GH influences apo B and apo E levels (34, 35) and hepatic triglyceride production (36, 37). In addition, GH may influence the ratio between apo B-100 and apo B-48 by an effect on apo B mRNA editing (38). Some of the above effects seem dependent on the pattern of GH secretion, and the continuous ("female") substitution used here in male rats may have different effects than a pulsatile ("male") administration pattern (34, 35).

Regardless of the above reservations, our results raise the question whether conditiohs with decreased GH secretion are associated with an increased sensitivity to dietary cholesterol. GH secretion in humans is reduced with age (28, 39), and concomitantly plasma LDL-cholesterol levels rise (40, 41). Whether the age-dependent GH reduction has a casual relation to the reduced clearance of plasma LDL that occurs with age in adults on a Western diet (42, 43) remains to be determined. If so, certain individuals with a particularly pronounced deficiency of GH with age might benefit from GH substitution (44). The administration of GH to adult patients with GH deficiency has been shown to lower plasma cholesterol levels (45). Studies on the plasma lipoprotein response to a challenge with dietary cholesterol in such individuals will be of interest.

In conclusion, the presence of GH appears to play an important role for maintaining the rat resistant to dietary cholesterol. Further studies are now needed to elucidate the biochemical pathways behind the apparent links between GH, hepatic LDL receptors, plasma cholesterol, and the development of atherosclerosis.

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