Mode of growth hormone administration influences triacylglycerol synthesis and assembly of apolipoprotein B-containing lipoproteins in cultured rat hepatocytes

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Abstract Hypophysectomized female rats were treated for 1 week with thyroxine (10 μg/kg · day), cortisol (400 μg/kg · day), and bovine GH (1 mg/kg · day) either as two daily subcutaneous injections (GH×2) or as a continuous subcutaneous infusion (GHc) in order to mimic the male and female specific GH secretory patterns, respectively. Hepatocytes were then isolated and kept in short-term cultures. Hypophysectomy decreased the synthesis of triacylglycerol. Treatment with GH×2 had no or small effects, while GHc normalized the effect of hypophysectomy. ApoB-100 VLDL was assembled before apoB-48 VLDL. ApoB-48 was first assembled as an HDL particle (apoB-48 "HDL"). Hypophysectomy decreased the proportion of intracellular apoB-48 that was recovered as VLDL. Moreover, the proportion of apoB-48 of total apoB in VLDL decreased. Only GHc fully restored the effect of hypophysectomy by inducing an 4-fold increase in the assembly of apoB-48 VLDL, while treatment with GH×2 gave rise to a 1.8-fold increase. Hypophysectomy resulted in a decrease in the proportion of apoB-48 that was secreted as VLDL and a decrease in the proportion of apoB-48 of total apoB in VLDL. Only treatment with GHc fully restored the secretion of apoB-48 VLDL by inducing an almost 4-fold increase in the secretion of apoB-48 VLDL, while the corresponding value for treatment with GH×2 was 1.7. However, GH×2 increased the proportion of the secreted apoB-48 that was recovered in VLDL to the levels found in normal rats and in rats treated with GHc, but this finding was due to a failure of GH×2 treatment to increase the secretion of apoB-48 "HDL". In summary, a continuous infusion of GH to hypophysectomized rats, mimicking the female secretion of GH, normalized the triacylglycerol synthesis and secretion as well as apoB-48 VLDL assembly and secretion to those levels observed in hepatocyte cultures from intact female rats.—**Sjöberg, A., J. Oscarsson, J. Borén, S. Edén, and S-O. Olofsson.** Mode of growth hormone administration influences triacylglycerol synthesis and assembly of apolipoprotein B-containing lipoproteins in cultured rat hepatocytes. *J. Lipid Res.* 1996. **37:** 275–289.

Supplementary key words pituitary • hypophysectomy • corticosteroids • thyroid hormones

Apart from its well-known effects on longitudinal bone growth, growth hormone (GH) decreases body fat mass (1, 2), increases adipose tissue lipolysis (3, 4), and influences serum lipoprotein concentrations in the rat (5–9).

ApoB is the essential apolipoprotein of the triacylglycerol- and cholesteryl ester-rich serum lipoproteins, chylomicrons, VLDL, IDL, and LDL. There are two forms of apoB, i.e., apoB-48 and apoB-100 (10). ApoB-100 consists of 4536 amino acids, whereas apoB-48 corresponds to the N-terminal 48% of apoB-100. Both proteins are coded for by the same gene and a posttranscriptional editing of the apoB-100 mRNA converts a Gln codon in position 2153 to a stop codon in the apoB-48 mRNA (11–13). The rat liver secretes both apoB-48 and apoB-100, but on separate VLDL particles

Abbreviations: apoB, apolipoprotein B; apoB-100 VLDL, VLDL containing apoB-100; apoB-48 VLDL, VLDL containing apoB-48; apoB-48 "HDL", the dense particle (banding in the HDL density range) containing apoB-48; apoE, apolipoprotein E; EMEM, Eagle's minimum essential medium; FCS, fetal calf serum; GH, growth hormone; GH×2, growth hormone given as two daily injections; GHc, growth hormone given as a continuous infusion; T_4, thyroxine; C, cortisol; HDL, high density lipoproteins; HX, hypophysectomized; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; SEM, standard error of the mean; SD, standard deviation; SDS, sodium dodecyl sulfate; SDS-PAGE, PAGE carried out in the presence of SDS; VLDL, very low density lipoproteins.

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(10, 14–16). We have demonstrated that GH regulates the hepatic expression of apoB-48 and apoB-100 by influencing the editing of apoB-100 mRNA (17, 18).

In recent years, evidence has accumulated on the role of the supply of fatty acids and the biosynthesis of triacylglycerol for the assembly of apoB-containing VLDL (19–27). In HepG2 cells, the assembly of apoB-100-containing VLDL occurred during translation/translocation of apoB-100, starting when the protein reached a size of about 80 kDa (28). During the assembly process there was a direct relation between the size of the nascent peptide and the triacylglycerol load of the assembled particle. Using a rat hepatoma cell line, apoB-48-containing VLDL has been shown to be assembled in two distinct steps (27). Thus, an apoB-48-containing HDL-like particle (apoB-48 "HDL") was shown to be the precursor of apoB-48-VLDL.

It is known that the hepatic synthesis and secretion of triacylglycerol is higher in female rats compared with male rats and dependent on gonadal steroids in vivo (29–32). Elam and co-workers have demonstrated that a continuous infusion of GH to hypophysectomized rats increased the synthesis of triacylglycerol (33) and secretion of VLDL (34). However, the secretion of GH has been shown to be sexually dimorphic in the rat. In male rats, GH is secreted episodically with very low or undetectable levels of GH between the pulses. In female rats, GH is secreted more continuously with irregular pulses (35). This sex difference in the secretory pattern of GH has been shown to be regulated by the gonadal steroids (35). Sex differences in the hepatic metabolism, including hepatic steroid metabolism, have been shown to be regulated by the sexually dimorphic secretory pattern of GH (35–37). Using a model to mimic the male and female specific secretory patterns of GH in hypophysectomized rats (35–38), we have demonstrated that the sex differences in serum HDL cholesterol and apoE concentrations were dependent on the more continuous secretion of GH in female rats (6–8). More recently, we have shown that treatment of hypophysectomized rats with a continuous infusion of GH, but not intermittent administration of GH, increased the secretion of apoE from cultured hepatocytes obtained from these animals (18).

In this paper we have taken these studies further by investigating the effects of continuous infusion and two daily injections of GH, to mimic the female and male specific secretory patterns of GH, respectively, on the assembly and secretion of apoB-48 and apoB-100-containing lipoproteins as well as on the triacylglycerol synthesis and secretion in primary cultures of rat hepatocytes. We present results indicating that the secretory pattern of GH influences the triacylglycerol synthesis and secretion as well as the assembly of VLDL, in particular the formation of apoB-48-containing VLDL.

MATERIALS AND METHODS

Materials

Bovine growth hormone (GH) was a generous gift from American Cyanamid Co. (Princeton, NJ); hydrocortisone phosphate (Solu Cortef®) was obtained from Upjohn (Puurs, Belgium); collagenase type IV, fatty acid-free bovine serum albumin, oleic acid (cell culture grade), phenylmethylsulfonyl fluoride (PMSF), and pepstatin A were purchased from Sigma Chemical Co. (St. Louis, MO); L-T_4 was from Nycomed (Oslo, Norway); Eagle's minimum essential medium (EMEM), Earl's balanced salt solution, Hank's balanced salt solution, nonessential amino acids, and HEPES were obtained from Flow Laboratories (Irwine, England); Eagle's minimum essential medium without methionine and glutamine was from GIBCO; [^{35}S]methionine, [^{3}H]glycerol, [^{14}C]methylated protein standards, [^{14}C]oleic acid (specific activity 50–60 mCi/mmol) and Amplify® were from Amersham International (Aylesbury, Buckinghamshire, United Kingdom); insulin (Actrapid; 100 U/ml) was obtained from Novo Industries (Copenhagen, Denmark); heparin was purchased from Lövens (Ballerup, Denmark); Immunoprecipitation was from Bethesda Research Laboratories (Gaithersburg, MD); all chemicals used for polyacrylamide gel electrophoresis were obtained from Bio-Rad (Richmond, CA). Trasylol (aprotinin) was from Bayer (Leverkusen, Germany). Calpain inhibitor I and calpain inhibitor II were purchased from Boehringer Mannheim (Mannheim, Germany). Pre-coated TLC plates with silica gel 60 were from Merck (Darmstadt, Germany). Triglycerides GPO-PAP, enzymatic colorimetric assay was from (Boehringer Mannheim, Germany).

Animals and hormonal therapy

Female Sprague-Dawley rats were obtained from Alab Laboratory Services (Stockholm, Sweden) and kept in a temperature (24–26°C)- and humidity (50–60%)-controlled vivarium in which the lights were on between 0500–1900 h. Water and standard laboratory chow (type R 34, Ewos, Södertälje, Sweden) were freely available. Hypophysectomy and hormonal therapies were performed as previously described (6, 17, 18). In short, hypophysectomy was carried out at 50 days of age by the parapharyngeal route (39). Hormonal therapies were started 7–10 days later. Hydrocortisone phosphate (C; 400 μg/kg • day) together with L-thyroxine (T_4; 10 μg/kg • day) were given as a daily subcutaneous (sc) injection at 0800 h. Bovine GH (GH; 1 mg/kg • day) was administered either continuously by means of an Alzet osmotic mini-pump 2001 (Alza Corp., Palo Alto, CA), which was implanted sc on the back of the rats, or as two daily sc

injections at 0800 and 2000 h. We used four or five experimental groups; normal rats (N), hypophysectomized rats (HX), or HX rats given T_4 and C (HX+T4C), hypophysectomized rats given T_4, C, and GH as continuous infusion (GHc), and hypophysectomized rats given T_4, C, and GH as two daily injections (GH×2). Hormonal treatment was given for 7 days. The weight gains observed were similar to those previously reported (6–8).

Cell culture, metabolic labeling, and isolation of microsomal lipoproteins

Rat hepatocytes were isolated and kept in short time culture as described previously (17), but the culture dishes were not coated with collagen (18, 40). An immunoradiometric assay for apoB was used to measure the accumulation of apoB in the culture medium during a 4-h incubation (17, 40). Labeling of the cells (7×10^6 cells/56 cm^2 culture dish) with [^{35}S]methionine for 2.5 h and chase with a surplus of cold methionine for 4 h were carried out as described previously (17). Pulse labeling of the cells with [^{35}S]methionine for shorter periods was carried out after 30 min pre-incubation in culture medium lacking cold methionine. The cells were then washed five times in 3 ml EMEM containing a surplus (11 mM) of cold methionine and chased in another 3 ml of this medium. The cells were harvested and homogenized in the presence of aprotinin (100 kallikrein inhibitor units/ml), leupeptin (0.1 mM), phenylmethylsulfonyl fluoride (1 mM), calpain inhibitor I (17 μg/ml), calpain inhibitor II (7 μg/ml), and pepstatin A (1 μM) as described before (17, 18, 41). The total microsomal fraction was isolated, disrupted by sodium carbonate, and the released material recovered as described earlier (41).

Sucrose gradient ultracentrifugation of the apoB-containing lipoproteins

The apoB-48 and apoB-100-containing lipoproteins secreted into the medium were analyzed by gradient ultracentrifugation as described earlier (18). The apoB-48 and apoB-100-containing lipoproteins recovered from the microsomal content of the hepatocytes were subjected to ultracentrifugation in a sucrose gradient formed by layering from the bottom of the tube; 2 ml 49% sucrose, 2 ml 25% sucrose, 5 ml sample in 10% sucrose, and 3 ml 0% sucrose. All solutions contained PBS (50 mM phosphate buffer, pH 7.4, containing 150 mM NaCl) supplemented with 5 mM EDTA, aprotinin (100 Kallikrein inhibitory units/ml), leupeptin (0.1 mM), PMSF (1 mM), calpain inhibitor I (17 μg/ml), calpain inhibitor II (7 μg/ml), and pepstatin A (1 μM). The gradients were ultracentrifuged at 35,000 rpm in a Beckman SW 40 rotor (Beckman Instruments, Fullerton, CA) for 65 h at 12°C and unloaded from the bottom into 11–13 fractions. ApoB-48 and apoB-100 were recovered from each of these fractions by immunoprecipitation (17, 42), followed by electrophoresis in 3–15% polyacrylamide gels containing sodium dodecyl sulphate (SDS). The bands corresponding to apoB-48 and apoB-100 were cut out of the gel and the radioactivity was counted after digestion of the gel (42). The radioactivity of the different apolipoproteins recovered from each fraction of the sucrose gradient was related to the DNA content of the cells in each culture dish (43).

Estimation of triacylglycerol biosynthesis rate and mass

To estimate the rate of synthesis of triacylglycerol, the incorporation of [^{14}C]oleic acid into triacylglycerol was measured. Hepatocytes (3.5×10^6 per 28 cm^2 culture dish) isolated from the different experimental groups were incubated for 0, 25, and 60 min with 6 μCi [^{14}C]oleic acid and 100 μM oleic acid complexed (44) to fatty acid-free bovine serum albumin (3 g/100 ml) in 1 ml medium containing 10% FCS. Lipids in the cells and the medium were extracted as described by Bligh and Dyer (45) and subjected to thin-layer chromatography in chloroform–acetic acid 96:4. The spot corresponding to triacylglycerol was identified after staining in iodine vapor, scraped off, extracted in 0.5 ml cyclohexane, and counted in a liquid scintillator. The radioactivity of the triacylglycerol recovered from each culture dish was related to the DNA content of the cells in the culture dish (43). The amount of triacylglycerol in the cells and the medium was estimated by measuring incorporation of [^{3}H]glycerol into triacylglycerol after 4 h incubation with [^{3}H]glycerol in EMEM with 10% FCS (40, 46). Different precursors ([^{3}H]glycerol, [^{14}C]oleic acid) were used in order to minimize the possibility that the differences between the various treatment groups reflected a difference in the consumption of the precursor through another metabolic pathway than triacylglycerol synthesis.

The mass of triacylglycerol in the medium was determined using an enzymatic colorimetric assay (40). In short, hepatocytes (7×10^6 cells/56 cm^2 culture dish) were incubated for 4 h with EMEM with 10% FCS. One ml medium from each of four dishes obtained from one rat was extracted separately according to Bligh and Dyer (45). The extracts were pooled and after evaporation to dryness under N_2 the lipids were dissolved in the buffer of the enzymatic colorimetric assay and triacylglycerol was determined according to the protocol of the manufacturer. In one control experiment, medium was obtained after the overnight incubation (15–16 h) of the hepatocytes with EMEM without FCS. The triacylglycerol content was determined as described above.

Statistical analysis

Values are expressed as means ± SEM. Comparisons between means were made by analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple range test between individual groups. The values were transformed to logarithms when appropriate.

RESULTS

Effects of hypophysectomy and hormonal therapy on the rate of triacylglycerol biosynthesis

The amount of radioactivity recovered in the triacylglycerol fraction (both cells and medium) after a 4-h incubation with [^{3}H]glycerol was used as an estimate of the amount of triacylglycerol that accumulated in the system during this period of time (**Fig. 1**). Using this method we could detect a 30% higher labeling of triacylglycerol (both cells and medium) in hepatocyte cultures from female rats compared to those from male rats (data not shown). The amount of labeled triacylglycerol decreased in the cells and medium after hypophysectomy and treatment with thyroxin (T_4) and cortisol (C). There

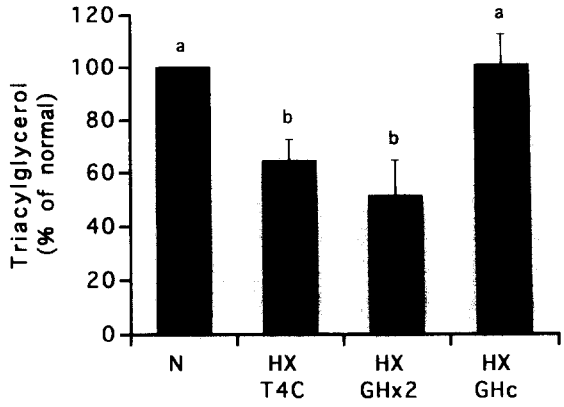


Fig. 1. The results of steady-state labeling of triacylglycerol with [^{3}H]glycerol for 4 h. The results presented are the sum of labeled triacylglycerol in the cells and medium. Hepatocytes were isolated (3.5 × 10^6 cells per 28 cm^2 culture dish) from normal female rats (N), hypophysectomized rats given T_4 and cortisol alone (HX;T4C), or in combination with GH administered either as two daily injections (GH×2), or as a continuous infusion (GHc). All incubations were performed in EMEM + 10% FCS. The cells and medium were recovered after each incubation period and extracted as described in Methods and Materials. The extracted lipids were recovered and chromatographed on high resolution thin-layer plates in chloroform–acetic acid 96:4 (v/v). The plates were stained with iodine and the spot corresponding to triacylglycerol was scraped off the plate into scintillation vials, 0.5 ml cyclohexane was added, and the radioactivity was counted. The results are expressed as the percentage of the activity observed in cells and medium derived from normal rats (N). Values are means ± SEM of (N), n = 7, (HX:T4C), n = 5, (GH×2), n = 3, and (GHc), n = 5 observations. Each observation represents a different liver perfusion and is the mean of the results obtained from five cell culture dishes. Values with different designations are significantly different from each other ($P < 0.01$).

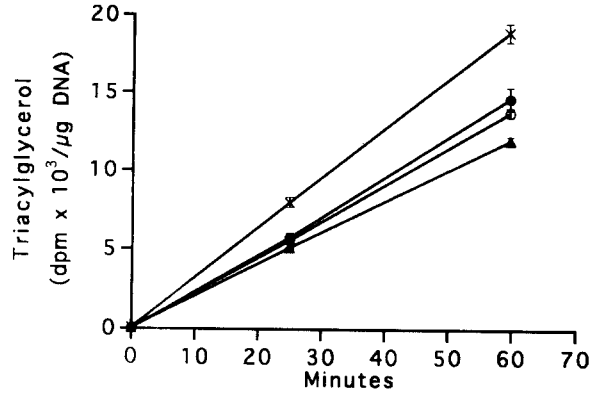


Fig. 2. The rate of incorporation of [^{14}C]oleic acid into triacylglycerol in cultured rat hepatocytes isolated from normal rats (N; ●), HX rats treated with T_4 and cortisol alone (HX + T4C; ▲), or in combination with GH given either as two daily sc injections (HX + GH×2; ○), or as a continuous infusion (HX + GHc, X). Rat hepatocytes (3.5 × 10^6 cells per 28 cm^2 culture dish) were incubated with 6 µCi [^{14}C]oleic acid (complexed to albumin) for 0, 25, and 60 min. The cells were recovered after each incubation period and extracted as described in Materials and Methods. The extracted lipids were recovered and chromatographed on high resolution thin-layer plates in chloroform–acetic acid 96:4 (v/v). The plates were stained with iodine and the spot corresponding to triacylglycerol was scraped off the plate into scintillation vials, 0.5 ml cyclohexane was added, and the radioactivity was counted. The values are from one representative experiment and each value is based on three analyses. The experiment was repeated twice with similar results. To obtain an estimate of the rate of the biosynthesis, the slopes of the curves obtained after the different treatments were calculated and related to that obtained for normal rats (which was set to 100%). The rate of the biosynthesis (as related to normal rats) was: Hx: 70%, GH×2: 76% and GHc: 104% (mean of three experiments).

was no effect of treatment with GH as two daily injections (GH×2), whereas treatment of the hypophysectomized rats with GH as a continuous infusion (GHc) increased the presence of labeled triacylglycerol in the cells and medium (Fig. 1).

The rate of incorporation of [^{14}C]oleate into triacylglycerol was investigated in isolated hepatocytes (**Fig. 2**). Cells from hypophysectomized rats treated with T_4 and C had a lower rate of incorporation of [^{14}C]oleate into triacylglycerol than cells from normal female rats. Treatment of hypophysectomized rats with GH×2 had small or no effects on the rate of incorporation, while treatment of the hypophysectomized rats with GHc increased the incorporation rate to levels that were similar or even above those of cells obtained from normal female rats (Fig. 2). The effect of the various treatment regimens on the rate of incorporation of [^{3}H]glycerol into the triacylglycerol fraction was similar (data not shown).

The accumulation of triacylglycerol in the medium was also measured as the mass of triacylglycerol after a 4-h incubation in EMEM with 10% FCS (**Fig. 3**). There was a significant decrease in the accumulation of triacylglycerol in the medium after hypophysectomy and treatment with T_4 and C. Treatment of the rats with GH×2

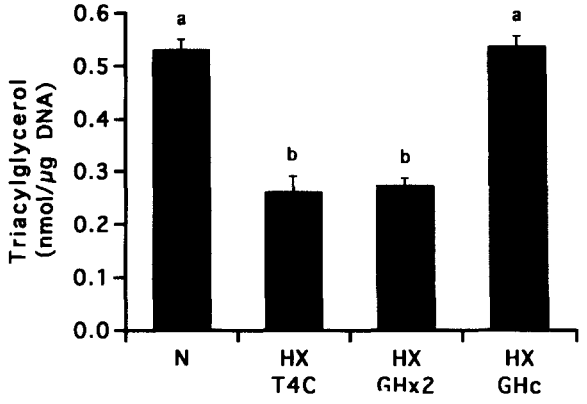


Fig. 3. Effects of hypophysectomy and hormonal treatment on the accumulation of triacylglycerol in the culture medium. Hepatocytes were isolated from normal female rats (N), hypophysectomized rats given T_4 and cortisol alone (HX), or in combination with GH administered as two daily injections (GHx2) or as a continuous infusion (GHc). The amount of triacylglycerol present in the medium (EMEM + 10% FCS) after a 4-h incubation was determined by extraction of lipids followed by determination of triacylglycerol with an enzymatic colorimetric assay (see Materials and Methods). The results are expressed as the means ± SEM of four observations. Each observation represents a different liver perfusion. Values with different designations are significantly different from each other ($P < 0.05$).

did not change the amount of triacylglycerol but there was a significant increase in the accumulation of triacylglycerol when the rats were treated with GHc. In a control experiment, the mass of triacylglycerol was measured in medium without FCS during the overnight incubation for 14–15 h. The amount of triacylglycerol present in medium of normal rats was 2.09 nmol/μg DNA, in the medium of hypophysectomized rats given T_4 and C 0.75 nmol/μg DNA, in the medium of rats given GHx2 0.43 nmol/μg DNA, and finally, in the medium from rats given GHc 1.16 nmol/μg DNA.

Pulse-chase experiments on the assembly of apoB-48 and apoB-100-containing lipoproteins

Hepatocytes from normal female rats or from hypophysectomized female rats given thyroxine and cortisol (T_4 and C) were labeled for 15 min with [^{35}S]methionine and chased with an excess of cold methionine for periods between 0 and 45 min. After each chase period, the microsomes were recovered and extracted with sodium carbonate. The sodium carbonate-released material was subjected to gradient ultracentrifugation and apoB-48 and apoB-100 were recovered from each fraction of the gradient and analyzed by SDS-polyacrylamide gel electrophoresis (**Fig. 4A** and **B**). Radioactive apoB-100 was present in VLDL as well as in more dense fractions after the 15 min pulse. In addition to the apoB-100-containing lipoproteins, there were nascent apoB polypeptides present in the density region of HDL (fractions 3–5) and LDL (fractions 6–8). The nascent polypeptides had disappeared after 15–30 min chase. There was a considerable accumulation of radioactive apoB-100 in VLDL during 30 min chase. There was also an increase in apoB-100 radioactivity in the more dense fractions, but the rate of accumulation and the total amount of radioactivity of full-length apoB-100 in the more dense fractions were less than in the VLDL fractions (Fig. 4A).

After the pulse period the major amount of apoB-48 was present in HDL-like particles (apoB-48 "HDL"), while virtually no radioactivity could be detected as apoB-48-containing VLDL. A significant amount of radioactivity was first seen in the VLDL fraction after 15 min chase (Fig. 4A). In contrast to apoB-100, the radioactivity of apoB-48 in VLDL still increased after 30 min chase, while the apoB-48 radioactivity in apoB-48 "HDL" had decreased significantly after 45 min chase (Fig. 4A).

Similar results were obtained when the experiments were carried out in hepatocytes isolated from hypophysectomized rats (Fig. 4B). The dominating apoB-48 radioactivity was recovered in apoB-48 "HDL" but, in contrast to intact rats, only small amounts of radioactive apoB-48 were present in the VLDL fraction after 30 and 45 min chase (Fig. 4B and **Fig. 5**).

Effect of hypophysectomy and hormonal therapy on apoB-48 and apoB-100-containing lipoproteins present in the lumen of the microsomal fraction of the cells

In the next set of experiments, we investigated the influence of GH on the assembly of the apoB-48 and apoB-100-containing lipoproteins. In these experiments (**Fig. 6** and **Table 1**), the cells were labeled with [^{35}S]methionine for 2.5 h, conditions that resulted in a steady-state labeling of the apoB-48 and apoB-100 pools in the cells (17). In agreement with the results from the pulse-chase studies, apoB-100 mainly occurred on VLDL particles but also on more dense particles (Fig. 6A).

The main apoB-48 radioactivity was present on denser particles, primarily banding in the HDL density range of the gradient. A substantial amount of apoB-48 radioactivity was also present in the VLDL fraction (Fig. 6A). The most prominent observation in hypophysectomized rats that had been treated with T_4 and C was a decrease in the content of apoB-48 in VLDL (Fig. 6B), which was reflected in a lower proportion of the total VLDL apoB that was accounted for by apoB-48, as well as in a decrease in the proportion of intracellular apoB-48 that was recovered in the VLDL fraction (Table 1). GH treatment, independent of the mode of administration, increased labeled apoB-48 within the VLDL fraction. However, the apoB-48 radioactivity present in the VLDL fraction increased 4-fold (4.16 ± 1.1 mean ± SD, n = 3) when the hypophysectomized rats were treated with

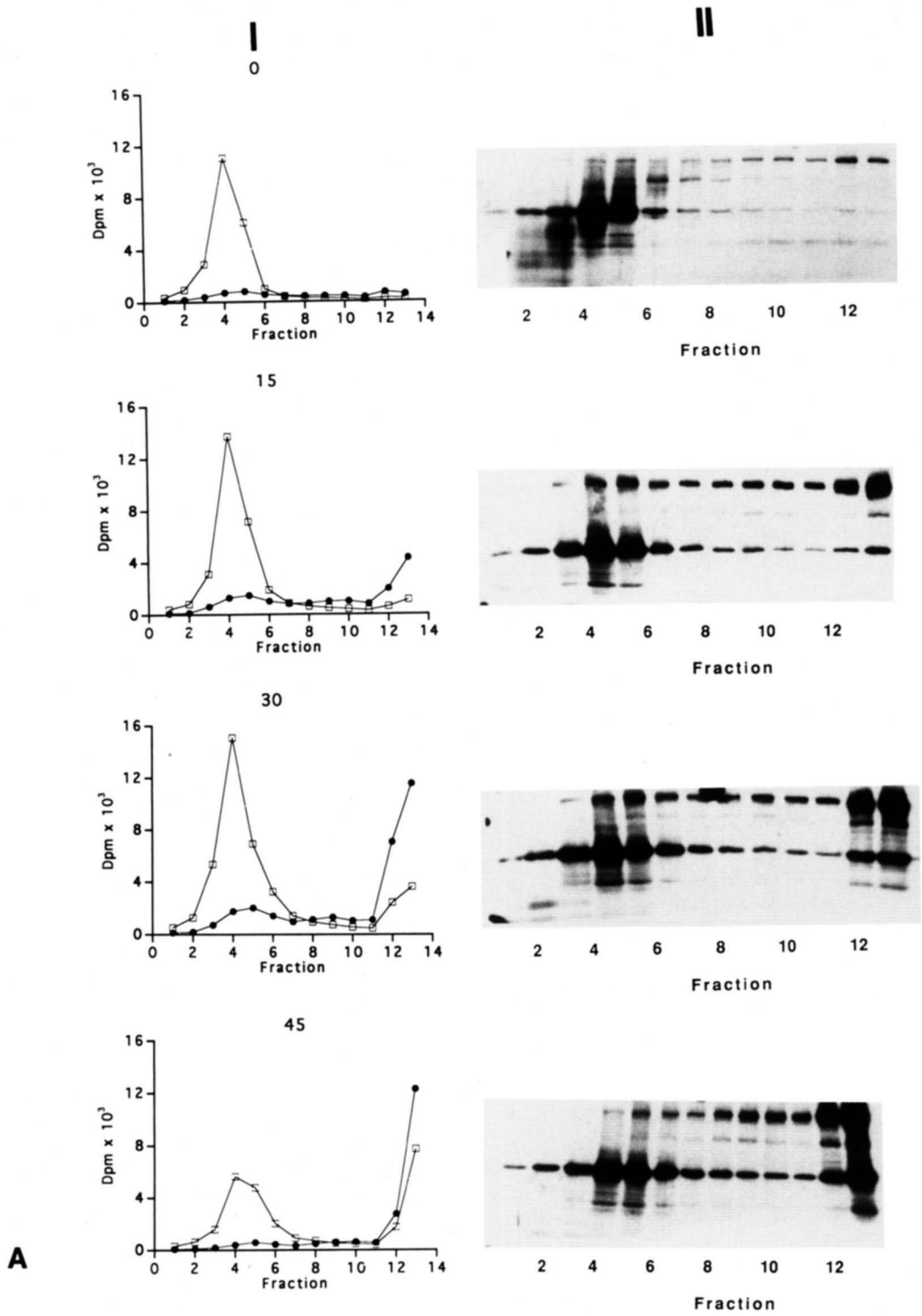
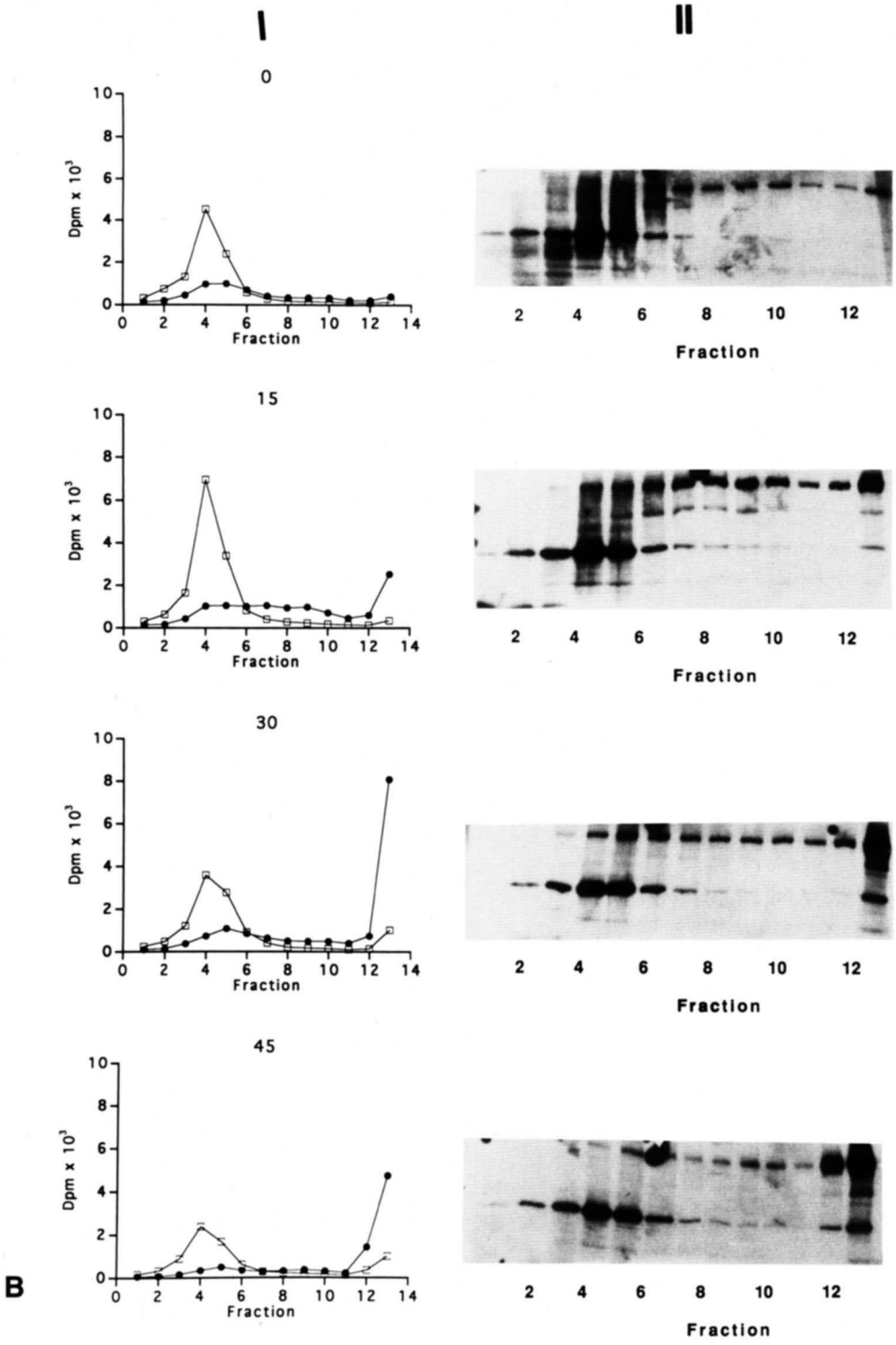


Fig. 4. Pulse-chase experiment on the assembly of apoB-48 (open squares) and apoB-100 (filled circles) containing lipoproteins in rat hepatocytes recovered from normal rats (A), HX rats treated with T_4 and cortisol (B). Rat hepatocytes (7×10^6 cells per 56 cm^2 culture dishes) were labeled for 15 min with 1 mCi [^{35}S]methionine and subsequently chased for 0, 15, 30, and 45 min with an excess of cold methionine. After each chase period, the total microsomal fraction was isolated and the luminal content was extracted by sodium carbonate. The sodium carbonate extracts were subjected to ultracentrifugation in sucrose gradients (see Material and Methods). ApoB-48 and apoB-100 were recovered from each fraction of the gradient by immunoprecipitation and electrophoresis in 3–15% polyacrylamide gradient gels containing SDS and the radioactivity was determined (A:I and B:I). A:II and B:II show the corresponding autoradiographs. The experiment was repeated twice with similar results.



GHc, while the corresponding increase after treatment with GH×2 was 1.77 ± 0.45-fold. There were no major differences in the effect of the different modes of GH administration on the increase in apoB-48 "HDL" radioactivity. Thus, after treatment with GHc, the apoB-48 radioactivity in the HDL density region increased 1.63 ± 0.12-fold (mean ± SD, n = 3) while the corresponding value for GH×2 was 1.48 ± 0.42. Together these results explained the observation that only GH given as a continuous infusion increased the proportion of intracellular apoB-48 that could be recovered in the VLDL fraction (Table 1). There were only relatively small effects of GH treatments on the intracellular apoB-100 radioactivity present in the VLDL fraction. Thus, treatment of the hypophysectomized rats with GH×2 gave rise to a 1.04 ± 0.16-fold increase in the apoB-100 radioactivity in VLDL (mean ± SD, n = 3), while the corresponding value for GHc was 1.29 ± 0.25. Moreover,

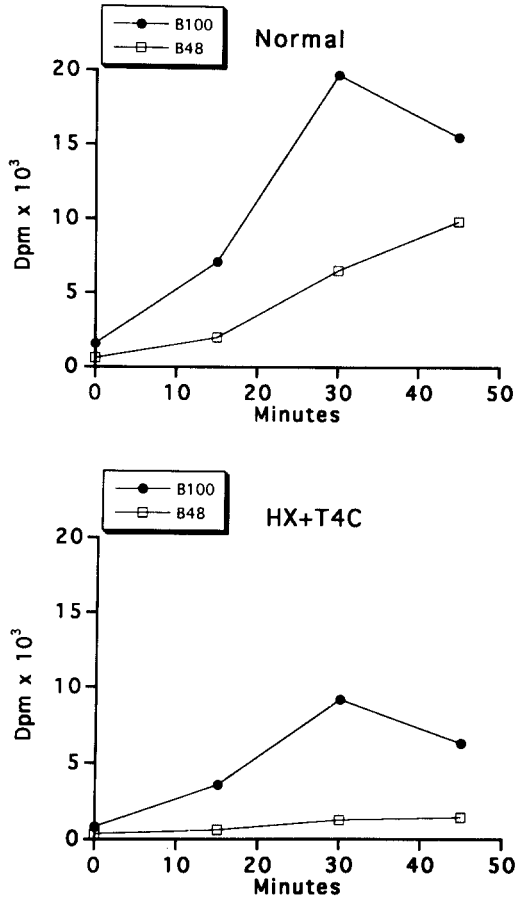


Fig. 5. The accumulation of radioactive apoB-48 and apoB-100 in the VLDL fraction as a function of the length of the chase period. Rat hepatocytes were pulse-labeled and chased as described in the legend to Fig. 4. After each chase period, the luminal content of the total microsomal fraction was isolated and ultracentrifuged as described in the legend to Fig. 4. ApoB-48 and apoB-100 were recovered from each fraction of the gradient and the radioactivity was determined (see legend to Fig. 4). As a function of the length of the chase period, the figure shows the accumulation of apoB-100 radioactivity as well as of apoB-48 radioactivity in the fractions of the gradient that contain VLDL.

there was no effect of hypophysectomy or GH treatment on the proportion of intracellular apoB-100 that was recovered in the VLDL fraction (Table 1).

Effect of hypophysectomy and hormonal therapy on the secretion of apoB-48 and apoB-100-containing lipoproteins

Rat hepatocytes in short time culture secreted both apoB-48 and apoB-100. Almost all apoB-100 was present in particles with a density of less than 1.006 g/ml, i.e., they belong to VLDL (**Fig. 7A**). ApoB-48, on the other hand, was secreted on both VLDL and as apoB-48 "HDL" (Fig. 7A). A larger proportion of apoB-48 was recovered in the VLDL fraction in the medium than in the secretory pathway (cf Table 1 and **Table 2**).

To estimate the rate of secretion of the assembled apoB-containing lipoproteins, the intracellular pool of lipoproteins was labeled to steady state (2.5 h incubation with [^{35}S]methionine). The labeled lipoproteins were chased with cold methionine in the medium for 4 h and analyzed by gradient ultracentrifugation.

ApoB-48-containing VLDL was the major VLDL species found in the medium of hepatocytes isolated from normal rats while hepatocytes isolated from hypophysectomized rats accumulated mainly radioactive apoB-100 in the VLDL fraction (Fig. 7A and B, Table 2). Treatment of the hypophysectomized rats with T_4 and C did not affect the relation between the two VLDL species in the medium (Fig. 7C). Thus, apoB-100 was still the major apoB species found in the VLDL fraction (Fig. 7B and C and Table 2). Treatment of the hypophysectomized rats with T_4 and C in combination with GHc or GH×2 significantly increased the content of labeled apoB-48 in the VLDL fraction (Fig. 7D and E). However, the increase in apoB-48 VLDL was larger when the hypophysectomized rats were treated with GHc than with GH×2. Thus, treatment with GHc gave rise to a nearly 4-fold (3.79 ± 1.14; mean ± SD, n = 3) increase in the apoB-48 radioactivity present in VLDL while the corresponding value observed after treatment with GH×2 was 1.69 ± 0.69 (mean ± SD, n = 3). In contrast to the observations in the secretory pathway, there was no difference between GH×2 and GHc treatments on the proportion of apoB-48 in the medium that was recovered in the VLDL fraction (expressed as the percentage of extracellular apoB-48 that was recovered in the VLDL fraction). This was due to a much larger increase in the secretion of apoB-48 "HDL" induced by GHc treatment. Thus, treatment with GHc gave rise to a 3-fold (3.24 ± 0.53; mean ± SD, n = 3) increase in the secretion of apoB-48 "HDL" while the corresponding value for GH×2 was 1.34 ± 0.23 (mean ± SD, n = 3). However, as in the cells, there was no difference between GH×2 and GHc treatments on the proportion of labeled apoB-48 in the VLDL fraction (percentage of total labeled apoB in VLDL). Also, as observed in the cells, the distribution of labeled apoB-100 in the different lipoproteins present in the medium was not affected by hypophysectomy or GH treatment (Table 2).

It appeared as if hypophysectomy and treatment with GH affected the total amount of labeled apoB-48 and apoB-100 in the medium (cf. Fig. 7). We therefore conducted experiments to measure the mass of total apoB present in the medium. The secretion of apoB from the cells measured as the accumulation of apoB in the culture medium during a 4-h incubation decreased after hypophysectomy and treatment with T4 and C (**Fig. 8**). Treatment of hypophysectomized rats with T4 and C together with GH×2 had no significant effect on

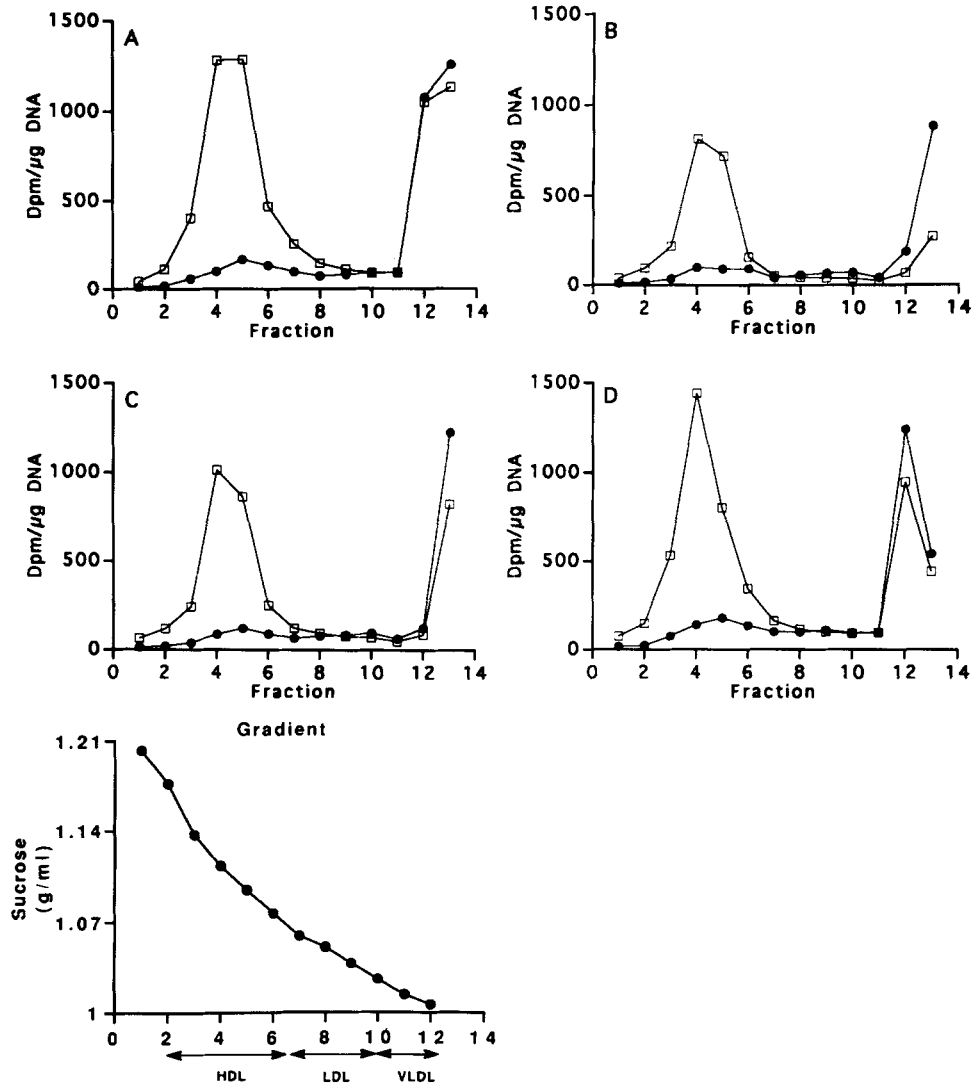


Fig. 6. Sucrose gradient ultracentrifugation of radiolabeled apoB-48 (open squares) and apoB-100 (filled circles) present in the luminal content of the total microsomal fraction of rat hepatocytes recovered from normal rats (A), HX rats treated with T_4 and cortisol alone (B), or in combination with GH given either as two daily sc injections (C) or as a continuous infusion (D). Rat hepatocytes (7×10^6 cells per 56 cm^2 culture dishes) were labeled for 2.5 h with 1 mCi [^{35}S]methionine. The total microsomal fractions were recovered and the luminal content was extracted by sodium carbonate. The sodium carbonate extracts were subjected to ultracentrifugation in sucrose gradients (see Material and Methods). ApoB-48 and apoB-100 were recovered from each fraction of the gradient by immunoprecipitation and electrophoresis in 3–15% polyacrylamide gradient gels containing SDS and the radioactivity was determined. HDL bands in fraction 2–7 (mean density is 1.090 g/ml), LDL bands in fraction 8–10 (mean density is 1.040 g/ml), and VLDL is present in the top fractions of the gradient. The values are from one representative experiment. The experiment was repeated three times with similar results.

the accumulation of apoB in the medium, while treatment of the hypophysectomized rats with T_4 and C in combination with GHc significantly increased the accumulation of apoB. However, no significant difference between the effects of GH×2 and GHc was observed (Fig. 8).

DISCUSSION

The present study demonstrates that GH is involved in the regulation of assembly and secretion of apoB, especially apoB-48 present in the VLDL fraction. These findings are an extension of our previous observations of an increased expression of apoB-48 and increased editing of apoB-100 mRNA after GH treatment of hypophysectomized rats (17). Moreover, the results of the present study indicated that the sexually dimorphic secretory pattern of GH affected the assembly of apoB-48-containing VLDL as well as the synthesis and secretion of triacylglycerol. This latter finding is an extension

TABLE 1. Effects of hypophysectomy and hormonal treatment on apoB-48 and apoB-100 distribution and composition of VLDL in the cells

Treatment	Proportion of ApoB-48 of Total ApoB in VLDL (%)	Proportion of ApoB-100 of Total ApoB in VLDL (%)	ApoB-48 in VLDL (% of total apoB-48 in the gradient)	ApoB-100 in VLDL (% of total apoB-100 in the gradient)
N	45.6 ± 7.3[a]	54.4 ± 7.3[a]	29.1 ± 3.4[a]	64.8 ± 4.6
HX	19.7 ± 4.8[b]	80.3 ± 4.8[b]	15.0 ± 0.7[b]	65.7 ± 1.7
HX + GH×2	33.7 ± 5.6[c]	66.3 ± 5.6[c]	17.9 ± 1.2[b]	67.7 ± 0.6
HX + GHc	42.4 ± 3.2[a]	57.6 ± 3.3[a]	28.6 ± 2.2[a]	70.5 ± 3.1

Female rats were hypophysectomized (HX) at 50 days of age and hormonal treatment was started 7–10 days later. All HX rats received T_4 and cortisol (C) as a daily sc injection. Bovine GH (1 mg/kg/day) was given either as two daily injections (GH×2) or as a continuous infusion (GHc). Hepatocytes were isolated and 7×10^6 cells were plated on 56 cm^2 culture dishes. The cells were recovered after 2.5 h incubation with [^{35}S]methionine. The total microsomal fractions were recovered and the luminal content was extracted by sodium carbonate. The sodium carbonate extracts were subjected to ultracentrifugation in sucrose gradients (see Materials and Methods). ApoB-48 and apoB-100 were recovered from each fraction of the gradient by immunoprecipitation and electrophoresis in 3–15% polyacrylamide gradient gels containing SDS and the radioactivity was determined. Values are means ± SEM, n = 3. Each observation is based on the results from one liver perfusion. In each column values with different superscripts are significantly different from each other ($P < 0.05$).

of previous observations by other investigators that GH given as a continuous infusion to hypophysectomized rats increased triacylglycerol synthesis in isolated hepatocytes (33) and VLDL triacylglycerol secretion in isolated perfused rat livers (34).

In previous reports, we have shown that certain aspects of lipoprotein metabolism are dependent upon the way GH is administered (6–8, 18). GH was given either as a continuous infusion or as two daily injections at 12-h intervals to mimic the sexually dimorphic secretory pattern of GH (6, 7, 18). This model has been extensively characterized in previous studies (36–38).

ApoB-100 did mainly assemble VLDL particles but apoB-100 also occurred in particles with higher density (banding in the HDL and LDL density regions of the gradient) in the cell. The presence of such particles have been demonstrated earlier in human (HepG2) (24) as well as in rat (McArdle RH7777 cells) hepatoma cells (27). In agreement with the observations made in previous studies (24), the majority of these dense particles were not secreted from the cells but appeared to be degraded. The observation that radioactive apoB-100 is present in the VLDL fraction already after the labeling period supports the possibility that this assembly process is closely coupled to the translation of apoB-100 (28). The first apoB-48-containing particle detected in the secretory pathway had the density of HDL (apoB-48 "HDL"), while the accumulation of apoB-48 in the VLDL fraction was delayed. These observations are in agreement with our previous findings (27) which indicate that the assembly of apoB-48 VLDL occurs in two steps (27), the first being the translation and translocation of apoB-48 and the formation of apoB-48 "HDL". A portion of the apoB-48 "HDL" pool in the cell was secreted but a large part of the pool of apoB-48 "HDL" could be retained in the cell serving as a precursor for the second step in the assembly process during which the major amount of lipids was added to the particle (27). The second step is highly dependent on the availability of fatty acids, indicating that the rate of biosynthesis of triacylglycerol is important for this step.

Hypophysectomy and GH treatment influenced the assembly of apoB-48-containing VLDL. There are two effects of GH that may be of importance for the understanding of the mechanism behind this influence of GH on the assembly of apoB-48 VLDL: *i)* GH increased the editing of apoB mRNA, thus promoting the biosynthesis of apoB-48 on behalf of apoB-100 (17); *ii)* GH influenced the rate of the biosynthesis of triacylglycerol (the present study). As both GH×2 and GHc increase the expression of apoB-48 and apoB-100 (18) while only GHc induces an increase in the biosynthesis of triacylglycerol, a rather complex influence of GH on the assembly process could be anticipated. The increased editing of apoB-100 mRNA induced by GH (17) is reflected in an increased biosynthesis and secretion of apoB-48 (17). It is likely that the first step of the assembly of apoB-48 VLDL is the formation of a lipid-poor precursor. This process may be dependent on the rate of biosynthesis of apoB-48, a circumstance that may explain the observation that treatment of the hypophysectomized rats with GH×2 or GHc resulted in the same increase in apoB-48 "HDL". Thus, treatment with GH (either GH×2 or GHc) provided virtually the same amount of precursor to the second step in the assembly process. The effect of a continuous infusion of GH on the biosynthesis of triacylglycerol would most likely have its main impact on the second step of the assembly as it has been demonstrated that this step is dependent on the availability of fatty acids (27), which most likely induces an increased rate of the biosynthesis of triacylglycerol. It could, therefore, be anticipated that treatment of the hypophysectomized rats with GHc would give rise to a larger increase in the rate of assembly of apoB-48 VLDL than treatment with GH×2. A 4-fold increase in apoB-48 in VLDL was indeed noticed when the rats were treated with GHc, while

treatment with GHx2 only resulted in a 1.8-fold increase. Moreover, treatment of the hypophysectomized rats with GHc gave rise to a normalization of the proportion of the intracellular apoB-48 that was recovered as VLDL, while GHx2 failed to normalize this proportion.

In summary, the observed influences of the different modes of GH administration on the assembly of apoB-48 VLDL closely followed what could be expected from the effects on the apoB mRNA editing and the rate of the biosynthesis of triacylglycerol.

The observed effect of GH treatment on the secretion of apoB-48 VLDL could be explained by the observed effects of GH on the assembly. Thus, treatment of the hypophysectomized rats with GHc gave rise to an almost 4-fold increase in the apoB-48 VLDL while the effect of

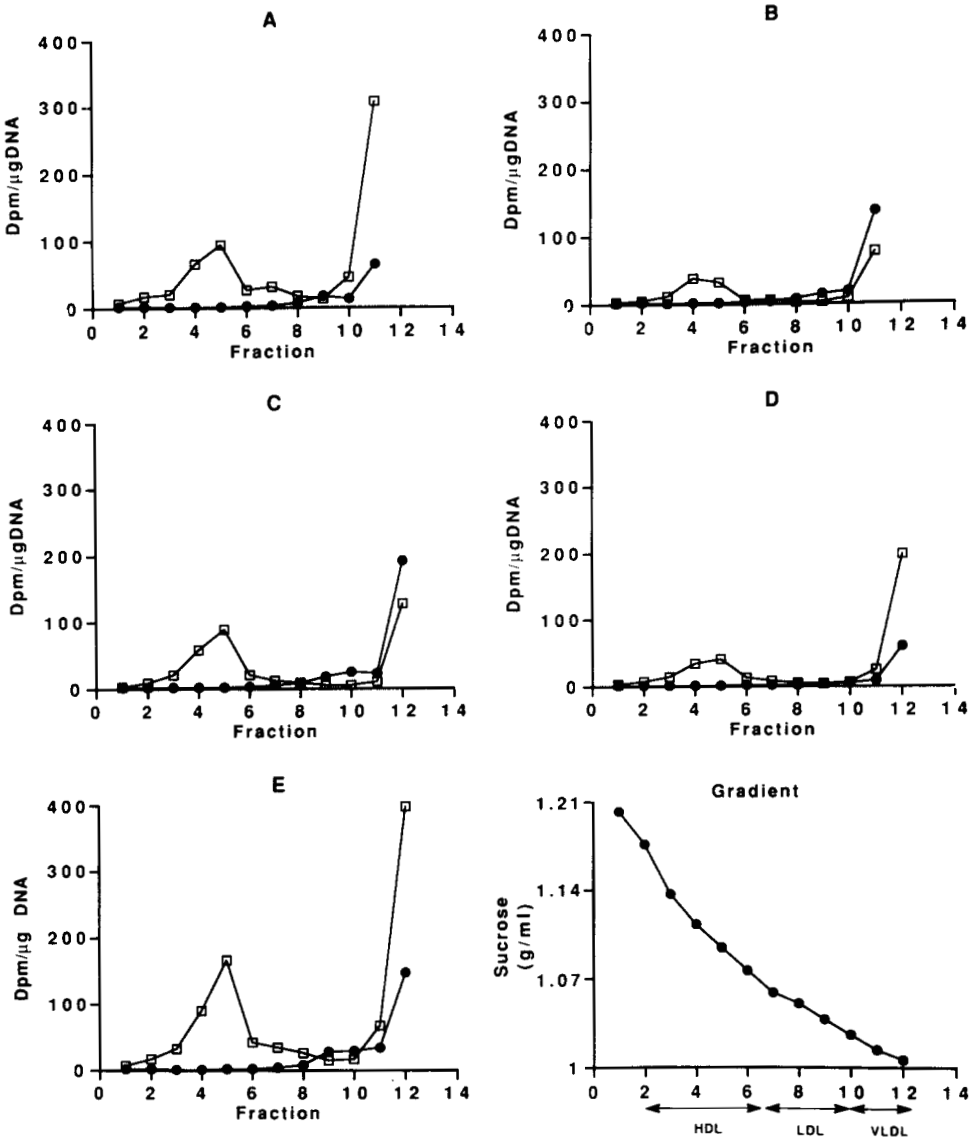


Fig. 7. Sucrose gradient ultracentrifugation of radiolabeled apoB-48 (open squares) and apoB-100 (filled circles) present in the medium of rat hepatocytes recovered from normal rats (A), HX rats (B), HX rats treated with T_4 and cortisol alone (C), or in combination with GH given either as two daily sc injections (D) or as a continuous infusion (E). Rat hepatocytes (7×10^6 cells per 56 cm^2 culture dishes) were labeled for 2.5 h with 1 mCi [^{35}S]methionine followed by a 4-h chase period with an excess of cold methionine. The chase mediums were subjected to ultracentrifugation in sucrose gradients (see Material and Methods). ApoB-48 and apoB-100 were recovered from each fraction of the gradient by immunoprecipitation and electrophoresis in 3–15% polyacrylamide gradient gels containing SDS and the radioactivity was determined. HDL bands in fraction 2–7 (mean density is 1.090 g/ml), LDL bands in fraction 8–10 (mean density is 1.040 g/ml), and VLDL is present in the top fractions of the gradient. The values are from one representative experiment. The experiment was repeated five times with similar results.

TABLE 2. Effects of hypophysectomy and hormonal treatment on apoB-48 and apoB-100 distribution and composition of VLDL in the medium

Treatment	Proportion of ApoB-48 of Total ApoB in VLDL	Proportion of ApoB-100 of Total ApoB in VLDL (%)	ApoB-48 in VLDL (% of total apoB-48 in the gradient)	ApoB-100 in VLDL (% of total apoB-100 in the gradient)
N	70.9 ± 3.3[a]	29.1 ± 3.3[a]	48.9 ± 3.6[a]	78.9 ± 4.7
HXC	27.5 ± 3.2[b]	72.5 ± 3.2[b]	32.4 ± 3.9[b]	77.9 ± 5.0
HX + GHx2	58.4 ± 6.6[c]	41.6 ± 6.6[c]	41.8 ± 6.4[c]	73.5 ± 5.6
HX + GHc	53.8 ± 5.0[c]	46.2 ± 5.0[c]	42.1 ± 4.5[c]	79.4 ± 3.9

Female rats were hypophysectomized (HX) at 50 days of age and hormonal treatment was started 7–10 days later. All HX rats received T_4 and cortisol (C) as a daily sc injection. GH (1 mg/kg/day) was given either as two daily injections (GHx2) or as a continuous infusion (GHc). Hepatocytes were isolated and 7×10^6 cells were plated on 56 cm^2 culture dishes. The mediums were recovered after 2.5 h incubation with [^{35}S]methionine followed by a 4-h chase period with an excess of cold methionine. The mediums were subjected to ultracentrifugation in sucrose gradients (see Materials and Methods). ApoB-48 and apoB-100 were recovered from each fraction of the gradient by immunoprecipitation and electrophoresis in 3–15% polyacrylamide gradient gels containing SDS and the radioactivity was determined. Values are means ± SEM, n = 5. Each observation is based on the results from one liver perfusion. In each column values with different superscripts are significantly different from each other ($P < 0.05$).

GHx2 was considerably smaller (1.7-fold). However, both GHx2 and GHc normalized the distribution of apoB-48 along the density spectrum of the secreted lipoproteins, while only treatment of the hypophysectomized rats with GHc normalized this distribution in the microsomal lumen. These discrepant results could be explained by a larger secretion of apoB-48 "HDL" after treatment of the hypophysectomized rats with GHc. The reason for this finding is unclear as very little is known about the regulation of the secretion of this lipoprotein. One possibility is that a posttranslational degradation (47–54) is involved in a modification of the amount of apoB-48 "HDL" that is secreted.

Triacylglycerol synthesis and secretion have been shown to be influenced by gonadal steroids (29, 32). The present findings suggest that the effects of gonadal steroids on triacylglycerol synthesis in the rat liver is mediated by changes in GH secretion (35). However, the mechanisms by which the "feminine" secretory pattern of GH affects triacylglycerol synthesis are still unclear. It has been observed that the uptake of FFA is enhanced in the female rat liver compared to the male liver (30), indicating that the secretory pattern of GH may regulate triacylglycerol synthesis and secretion by influencing this process.

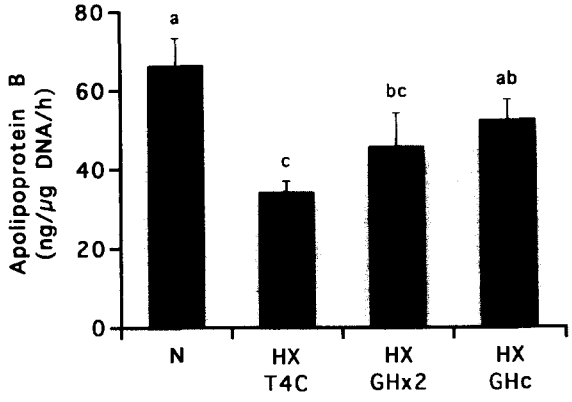


Fig. 8. Effects of hypophysectomy and hormonal treatment on the accumulation of apoB in the culture medium. Hepatocytes were isolated from normal female rats (N), hypophysectomized rats given T_4 and cortisol alone (HX + T4C), or in combination with GH administered as two daily injections (GHx2) or as a continuous infusion (GHc). The amount of apoB present in the medium (EMEM + 10% FCS) after a 4-h incubation was determined by an immunoradiometric assay. The values are means ± SEM of five observations. Each observation is from a different liver perfusion and based on the mean of three analyses. Values with different designations are significantly different from each other ($P < 0.05$).

The accumulation of apoB in the medium has been suggested to represent apoB secretion as our previous results indicated that no degradation of newly secreted apoB occurs during a 4-h incubation in the presence of 10% FCS (17). The effects of hypophysectomy and GH treatment on total apoB secretion appear to contradict our earlier reports that hypophysectomy or hormonal treatment does not influence the total apoB secretion (17). We later observed that total apoB determined in the medium increased substantially when the hepatocytes were cultured directly on the plastic dishes without collagen-coating (40). However, the relation between apoB-48 and apoB-100 in the medium did not differ between cultures with or without collagen-coating (40). We therefore believe that the present results are more accurate regarding the effects of hypophysectomy and GH treatment on total apoB secretion of hepatocytes.

In a previous paper, we have demonstrated that the secretory pattern of GH influences the hepatic secretion of apoE. It was demonstrated that a continuous infusion in contrast to two daily injections increased the total secretion of apoE as well as the apoE content of VLDL in the medium (18). It has been shown that stimulated VLDL secretion is associated with increased secretion of larger VLDL particles with an increased content of apoE (55). Moreover, the developmental increase in triacylglycerol secretion is in parallel with an increased

expression of apoB-48 in the rat (56). In line with these studies, an increased triacylglycerol synthesis and enhanced apoB-48-VLDL assembly were demonstrated in the present study. It could be speculated that the formation of a VLDL particle with the smaller apoB molecule allows a larger incorporation of apoE into the surface of this particle.

The obtained results do not lend any direct explanation to the decreased levels of apoB seen in serum after GH treatment of hypophysectomized rats (6). Because GH increased the secretion of total apoB from isolated hepatocytes, it is not likely that the decrease seen in the serum levels of apoB after treatment of hypophysectomized rats with GH×2 or GHc could be explained by an effect of GH on the rate of biosynthesis and secretion of apoB in the liver. Rather, the effects of GH on the serum levels may be due to the influence of the hormone on the degradation of the apoB-containing lipoproteins. This degradation involves, for example, the action of lipoprotein lipase. It has been shown that the activity of postheparin plasma lipoprotein lipase is increased by GH treatment of hypophysectomized rats (57). Moreover, the hepatic expression of the LDL receptor has also been shown to be regulated by GH (9). However, there is a possibility that the effect of GH on the type of apoB molecule that is synthesized (i.e., apoB-48 or apoB-100) could be of importance for the decreased levels of serum apoB. Thus, it has been demonstrated that apoB-48-containing lipoproteins have a more rapid turn-over in plasma than apoB-100-containing lipoproteins (15, 16). An increased secretion of apoB-48-VLDL may therefore result in decreased serum levels of apoB. Indeed, evidence has been presented that there is a negative relation between the degree of hepatic editing of apoB-100 mRNA and the serum apoB concentrations in various species (58).

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