Dramatically Increased Intestinal Absorption of Cholesterol Following Hypophysectomy Is Normalized by Thyroid Hormone

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Background & Aims: **Hypopituitarism is associated with dyslipidemia, and feeding hypophysectomized rats cholesterol induces severe hypercholesterolemia. This study aimed to unravel further how hypophysectomy alters cholesterol and bile acid metabolism.** ***Methods:*** **Intact and hypophysectomized rats were studied during challenge with dietary cholesterol and ezetimibe and upon hormonal substitution with growth hormone, cortisone, and thyroid hormone.** ***Results:*** **Five findings were established in hypophysectomized rats: (1) The intestinal absorption of cholesterol is doubled. (2) Treatment with ezetimibe abolishes the increases in serum and liver cholesterol. (3) Only thyroid hormone treatment normalizes the increased absorption of cholesterol. (4) The intestinal gene expression of cholesterol transporters NPC1L1 and ABCG5/G8 is unaltered, whereas the hepatic expression of ABCG5/G8 is diminished but strongly stimulated by thyroid hormone. The latter mechanism was supported by measurements of biliary cholesterol and of fecal neutral steroids. (5) The reduced hepatic expression of ABCG5/G8 and Cyp7a1 was normalized by cholesterol feeding, suggesting that other nonestablished mechanisms under pituitary control are important to maintain rats resistant to dietary cholesterol.** ***Conclusions:*** **The intestinal absorption of dietary cholesterol is under pituitary control largely exerted by thyroid hormone. Hepatic secretion of cholesterol and ABCG5/G8 expression are strongly stimulated in hypophysectomized rats during treatment with thyroid hormone.**

Hypopituitarism is associated with obesity and dyslipidemia.[1,2] In hypophysectomized (Hx) rats, plasma low-density lipoprotein (LDL) cholesterol is increased, whereas high-density lipoprotein (HDL) cholesterol is reduced.[3,4] The fecal excretion of bile acids and hepatic bile acid and cholesterol synthesis are reduced, as is the hepatic expression of LDL receptors (LDLRs) and activity of the rate-limiting enzyme in bile acid production cholesterol 7α-hydroxylase (Cyp7a1).[5,6] Hx rats are sensitive to dietary cholesterol, and feeding cholesterol increases plasma cholesterol 3- to 8-fold, whereas hepatic LDLRs are reduced.[4] Conversely, normal rats are resistant to dietary cholesterol.[4] The changes following Hx can be partially normalized by growth hormone (GH) substitution.[4–7] Also Hx-mice have elevated LDL cholesterol and a suppressed Cyp7a1 enzymatic activity.[8] However, while Hx mice are also sensitive to dietary cholesterol this cannot be explained by a faulty regulation of Cyp7a1 since in cholesterol-fed Hx mice Cyp7a1 is stimulated up to the same level as in normal mice.[8]

We here further unravel how hypophysectomy alters cholesterol and bile acid metabolism in rats. We found that the intestinal absorption of dietary cholesterol is increased in Hx rats, which is normalized by thyroid hormone treatment partly mediated by the hepatobiliary cholesterol export pumps ABCG5/G8 that are strongly stimulated when Hx rats receive thyroid hormone.

BASIC–ALIMENTARY TRACT

Materials and Methods

Animals

Fifty intact and 141 Hx male Sprague–Dawley rats (Taconic A&B, Ry, Denmark) were used in 6 experiments. Hx was performed at Taconic Laboratories on 200-g weight animals, and failure to gain weight was monitored. Animals had free access to water and chow, standard or enriched with 0.4% or 2% cholesterol/10% corn oil ± ezetimibe (EZE) (3 mg/kg/day; Ezetrol MSD-SP Limited, Hoddesdon, United Kingdom), for 1 week. T4 (L-Thyroxine; Sigma-Aldrich, St Louis, MO) and cortisone (Solu-Cortef, Pfizer, Sollentuna, Sweden) were injected subcutaneously (42 μg/kg/day and 400 μg/kg/day, respectively) every morning for 1 week. Bovine GH (from Dr A.F. Parlow, National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) was infused (1.5 mg/kg/day) by implanted pumps[7] (Alzet model 2ML1; Palo Alto, CA). Lights were on from 6 AM to 6 PM.

Rats were killed by decapitation during isoflurane anesthesia, and trunk blood was collected. Serum,

Abbreviations used in this paper: C4, 7α-hydroxy-4-cholesten-3-one; Cyp7a1, cholesterol 7α-hydroxylase; EZE, ezetimibe; FPLC, fast-performance liquid chromatography; GH, growth hormone; Hx, hypophysectomized; LDL, low-density lipoprotein; LDLR, LDL receptor; NPC1L1, Niemann-Pick C1-like 1 protein; T4, L-Thyroxine.

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liver, and proximal and distal small intestine were frozen in liquid nitrogen and stored at −80°C. The study was approved by the Institutional Animal Care and Use Committee.

Lipid Assays

Serum total cholesterol and triglycerides were determined using a Monarch Automated Analyzer (ILS Laboratories Scandinavia AB, Stockholm, Sweden). Hepatic total cholesterol and triglycerides were measured using kits (Roche Diagnostics GmbH, Mannheim, Germany). Lipids were extracted from 50 mg liver homogenates with chloroform/methanol (2:1, vol/vol) according to Folch et al[9] modified to contain Triton X-100.[10] Biliary cholesterol was determined by gas-liquid chromatography mass spectrometry[11] from 25 μL bile. Size fractionation of serum lipoproteins by fast-performance liquid chromatography (FPLC) was performed using 10 μL of serum from each individual as described.[12]

Cholesterol Absorption and Secretion

Cholesterol absorption was measured by the fecal dual-isotope method. At 9 PM, each rat received 0.5 mL corn oil by gastric gavage containing 5 μCi [^{14}C]-cholesterol (Amersham, Uppsala, Sweden) and 2 μCi [5,6-^{3}H]-β-sitostanol (American Radiolabeled Chemicals Inc).[13] Individual 24-hour stool collections were homogenized in phosphate-buffered saline (PBS), and 1 mL homogenate was extracted. The dosing mixture and fecal ^{14}C/^{3}H ratios were counted (disintegrations per minute), and percentage cholesterol absorbed was calculated/animal.[13–15]

Serum sitosterol and campesterol reflecting cholesterol absorption[15,16] were extracted from 10 μL/rat in duplicate. Derivatized samples were analyzed by gas chromatography mass spectrometry[17] using D5-campesterol/sitosterol as internal standard.

Biliary cholesterol secretion was determined during hypnorm/dormicum anesthesia by a 45-minute collection of bile from a catheter in the hepatic duct. To maintain temperature, animals were placed on heated pads.

Cholesterol and Bile Acid Synthesis

Enzymatic activity of microsomal HMG-CoA reductase (HMG-CoAR) and Cyp7a1 were assayed from the conversion of [^{14}C]HMG-CoA to mevalonate[18] (pmoles/mg protein/min) and from the formation of 7α-hydroxycholesterol (pmoles/mg protein/min) from endogenous microsomal cholesterol using isotope dilution-mass spectrometry[19] in duplicate. 7α-hydroxy-4-cholesten-3-one (C4) was assayed in 200 μL serum/animal as described.[20] C4 levels (ng/mL).

Protein Expression of Cholesterol 7α-Hydroxylase and β-Actin

Hepatic membranes[5] (50 μg) from each individual were electrophoresed on 6% SDS-PAGE, electrotransferred to a nitrocellulose filter, and probed against Cyp7a1 as described.[21] After stripping filters, β-actin was probed with the same protocol using as primary antibody against β-actin (ab8227; rabbit polyclonal diluted 1:10,000) and as secondary antibody a peroxidase-conjugated goat anti-rabbit immunoglobulin (ab6721; diluted 1:5000). Antibodies were from Abcam, Cambridge, United Kingdom. The ratio for Cyp7a1/β-actin was calculated for each individual. Western blots were repeated with the same results.

Gene Expression

Total RNA was extracted with Trizol reagent (Invitrogen) from livers or intestines pulverized in liquid nitrogen and DNase-treated with RQ1 DNase (Promega). Complementary DNA (cDNA) synthesis was performed using SuperscriptII (Invitrogen) or Omniscript (Qiagen). Quantitative real-time PCR was performed with TaqMan (ABI, Foster City, CA) or SYBR Green (Medprobe AS, Oslo, Norway) assay on an ABI Prism 7700 Sequence Detection System (ABI). 18S was used as endogenous control. Primers/probes were designed using Primer Express Software 2.0 (ABI) and are available on request.

Fecal Cholesterol and Neutral Steroids

Fecal homogenates were dried, and 2 × 50 mg were homogenized in water, and hydrolyzed for 1 hour in 1 mol/L NaOH in 90% ethanol at 67°C. Neutral sterols were extracted with cyclohexane. Samples were derivatized with trimethylsilane reagent (pyridine/hexamethyldisilane/trimethylchlorodisilane, 3:2:1, vol/vol/vol), solubilized in n-decane, and subjected to gas chromatography (30-m HP-5 column, part No. 19091J-413; Hewlett-Packard). 5α-cholestane and hyodeoxycholic acid were used as internal standards (50 μL/sample).

Statistical Analysis

Data show means ± SEM. The significance of differences between groups was tested by 1-way ANOVA, followed by post hoc comparisons according to Tukey using GraphPad Prism Software (San Diego, CA). Data were log transformed when there was a positive correlation between group means and group SD. For the experiment in Figure 2 (CTRL vs Hx rats), statistical significances between groups were tested by Student *t* test.

Results

Our initial hypothesis was that a reduced Cyp7a1 enzymatic activity[6] may explain why Hx rats are sensitive to dietary cholesterol. For this purpose, intact and Hx rats received chow ± 2% cholesterol/10% corn oil (cho-