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FARNESOID X RECEPTOR (FXR) IS A BILE ACID RECEPTOR THAT MEDIATES TRANSCRIPTIONAL REGULATION OF THE CHOLESTEROL 7α-HYDROXYLASE GENE (CYP7A1) BY BILE ACIDS John Yl Chiang, Rhonda Kimmel, Northeastern Ohio Univ 's Coll of Medicine, Rootstown, OH; Cary Weinberger, National Institute of Environmental Health Sci, Research Triangle, NC; Diane Stroup, Northeastern Ohio Univ 's Coll of Medicine, Rootstown, OH

Cholesterol 7α-hydroxylase catalyzes the rate-limiting step of the conversion of cholesterol to bile acids. The gene CYP7A1 is negatively regulated by bile acids at the gene transcriptional level. Two bile acid response elements (BARE) containing hormone response elements (HRE) have been mapped previously and a receptor-mediated mechanism was proposed to regulate CYP7A1 gene by bile acids. Deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) strongly activated FXR/RXR and stimulated the reporter activity from a heterologous promoter containing multiple copies of a FXR binding element in CHO cells. The effects of RXR/FXR on transcriptional activity of the rat and human CYP7A1/luciferase genes were studied in transient transfection assays in HepG2 cells. RXR/FXR stimulated rat CYP7A1 promoter/luciferase reporter activity and addition of DCA or CDCA strongly inhibited the promoter activity. Cotransfection with RXR/FXR reduced the IC_{50} of CDCA required for the inhibition of CYP7A1 promoter activity by 10-fold. Deletion analysis revealed that BARE-II was involved in RXR/FXR-mediated bile acid response. Electrophoretic mobility shift assay (EMSA) identified the direct repeat of HRE separated by 4 bases (DR4) in BARE-I as a RXR/FXR binding site in the rat but not human CYP7A1 gene and RXR/FXR did not bind to BARE-II. These results suggest that RXR/FXR act as a bile acid receptor and down regulate CYP7A1 gene transcription by interfering with the transcription factors that bind to BAREs.

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CAFESTOL, THE CHOLESTEROL-RAISING FACTOR IN BOILED COFFEE, INCREASES SERUM CHOLESTEROL LEVELS IN APOLIPOPROTEIN E*3-LEIDEN TRANSGENIC MICE BY SUPPRESSION OF BILE ACID SYNTHESIS Sabine M Post, Gaubius Lab, TNO-PG, Leiden Netherlands; Baukje De Roos, Martijn Vermeulen, Lydia Afman, Wageningen Agricul Univ, Wageningen Netherlands; Miek C Jong, Vivian Eh Dahlmans, Louis M Havekes, Gaubius Lab, TNO-PG, Leiden Netherlands; Frans Stellaard, Acad Hospital Groningen, Groningen Netherlands; Martijn B Katan, Wageningen Agricul Univ, Wageningen Netherlands; Hans Mg Princen, Gaubius Lab, TNO-PG, Leiden Netherlands

Cafestol, a diterpene present in unfiltered coffee brews, potently increases serum cholesterol levels in humans. So far, no suitable animal model has been found to study the biochemical background of this effect. We determined the effect of cafestol on serum cholesterol and triglycerides in different strains of mice and studied subsequently the mechanism of action in apoE*3-Leiden transgenic mice. ApoE*3-Leiden, LDLR+/- or WT mice were fed a high (0.05% w/w) or low (0.01% w/w) cafestol diet or a placebo diet for 8 weeks. In apoE*3-Leiden mice, serum cholesterol was increased by 3.46 mmol/L (95%CI [1.62;5.30]) on the low and by 6.35 mmol/L (95%CI [4.47;8.22]) on the high cafestol diet. In LDLR+/- and WT mice, the increases were 0.85 mmol/L (95%CI [-0.25;1.94]) and 0.62 mmol/L (95%CI [0.34;0.90]), respectively, on the low cafestol diet, and 2.37 mmol/L (95%CI [0.73;4.01]) and 1.21 mmol/L (95%CI [0.92;1.21]), respectively, on the high cafestol diet. The increase in total cholesterol was mainly due to a rise in VLDL and IDL cholesterol in all three mice strains. To investigate the mechanism of the cholesterol-raising effect, apoE*3-Leiden mice were fed a high cafestol or a placebo diet for 3 weeks. Cafestol suppressed enzyme activity and mRNA levels of cholesterol 7α-hydroxylase by 57% and 58% (both $p<0.05$), respectively. mRNA levels of enzymes involved in the alternative pathway of bile acid synthesis i.e. sterol 27-hydroxylase and oxysterol 7α-hydroxylase were reduced, by 32% ($p<0.05$) and 48% ($p<0.005$), respectively. The total amount of bile acids secreted in feces was decreased by 41%. Cafestol did not affect hepatic free and esterified cholesterol, but it decreased LDLR mRNA levels by 37% ($p<0.05$). VLDL particles contained a three times higher amount of cholesteryl esters, indicative for the secretion of a ß-VLDL-like particle. This was confirmed by a decreased VLDL triglyceride production in mice treated with cafestol (35.1±13.8 µmol/h/kg) compared to placebo treatment (63.1±17.5 µmol/h/kg) as a result of a reduction in hepatic triglyceride content by 52% ($p<0.05$). In conclusion, cafestol increases serum cholesterol levels in apoE*3-Leiden transgenic mice by suppression of the major regulatory enzymes in the bile acid synthesis pathways, leading to decreased LDLR mRNA levels and increased secretion of cholesterol esters by the liver. In analogy, we suggest that suppression of bile acid synthesis may provide an explanation for the cholesterol-raising effect of cafestol in humans.

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CHOLESTEROL 7α-HYDROXYLASE (CYP7A) GENE KNOCKOUT RESULTS IN HYPERCHOLESTEROLEMIA, GENDER-SPECIFIC ALTERATION IN BILE ACID POOL SIZE AND COMPOSITION AND IN GENDER-SPECIFIC HYPERINSULINEMIA. Sandra K Erickson, Univ of CA, San Francisco, San Francisco, CA; Steven R Lear, Univ of CA, San Francisco, CA; Ashok K Batta, Veterans Affairs Med Ctr, East Orange, NJ; Sarah Shefer, Univ of Medicine and Dentistry of New Jersey, Newark, NJ; Patricia J Blanche, Lawrence Berkeley National Lab, Berkeley, CA; Gerald Salen, Univ of Medicine and Dentistry of New Jersey, Newark, NJ; Ronald M Krauss, Lawrence Berkeley National Lab, Berkeley, CA

Cholesterol 7α-hydroxylase (cyp7A) is an hepatospecific enzyme that is proposed to play a key role in maintenance of cholesterol and bile acid homeostasis. It has been implicated in the etiologies of atherosclerosis and cholesterol gallstone disease. To study the role of cholesterol 7α-hydroxylase in the maintenance of lipid homeostasis, we bred out a mouse cyp7A gene knockout line from the line originally described (Ishibashi, et al. J. Biol. Chem. 271:18017-18023, 1996) and deposited in Jackson Labs. As expected, our line lacks cholesterol 7α-hydroxylase activity and contains the same altered cyp7A gene as that in the originally described line. However, in contrast to the originally described line, our line shows high pup survival when the mothers are maintained on a chow diet. In addition, our line showed no change in the % hyodeoxycholate content of gall bladder bile acids compared with that of their wild type genetic controls. In further contrast, in our line, both male and female cyp7A gene knockout mice were hypercholesterolemic compared with wild type: 166±14 mg cholesterol/dl vs 107±9 mg cholesterol/dl, p(0.003,respectively for the males and 140±10 mg cholesterol/dl vs 95±12 mg cholesterol/dl, p(0.01, respectively for the females. Analyses of plasma by gradient gel electrophoresis followed by oil red O staining showed that the major difference in the lipoprotein patterns in the cyp7A gene knockout mice compared with their wild type control was in the distribution of low density lipoprotein (LDL) particles. Relative to their wild type genetic controls, the smallest LDL-size particles (IVA+IVB) increased 56% in male cyp7A gene knockouts, p(0.002, and 83% in female cyp7A gene knockouts,p(0.001, while the largest size LDL particles (I and IIA+IIB) were reduced 33% in both, p(0.001. All cyp7A gene knockouts were normotriglyceridemic, normoglycemic and showed no change in plasma free fatty acids relative to wild type; however, male cyp7A gene knockouts were hyperinsulinemic relative to wild type, 3.28±0.53µg insulin/l vs 0.95±0.30 µg insulin/l,respectively, p(0.04, suggesting that they had developed insulin resistance. Total fecal bile acids were decreased 75% in male cyp7A gene knockouts and 56% in female knockouts relative to wild type. Gall bladder bile acid distribution in male cyp7A gene knockouts showed 41% decrease in cholate, p(0.002, and 2.2 fold increase in β-muricholate, p(0.002, relative to that in wild type. In contrast, female cyp7A gene knockout gall bladder bile acid distribution showed little change from that in wild type. The data taken together support a pivotal role for cholesterol 7α-hydroxylase in maintenance of plasma cholesterol and lipoprotein homeostasis. In addition, the data suggest the possibility that cholesterol 7α-hydroxylase and/or its products and/or bile acids affect insulin homeostasis in a gender-specific fashion.

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FIBRATES SUPPRESS BILE ACID SYNTHESIS VIA PPARα-MEDIATED DOWN-REGULATION OF CHOLESTEROL 7α-HYDROXYLASE GENE EXPRESSION. Sabine M Post, Gaubius Lab, TNO-PG, Leiden Netherlands; Bart Staels, Inst Pasteur de Lille, Lille France; Emma De Fabiani, Institute of Pharmacological Sci, Univ of Milan, Milan Italy; John Yl Chiang, Northeastern Ohio Univ 's Coll of Medicine, Rootstown, OH; Hans Mg Princen, Gaubius Lab, TNO-PG, Leiden Netherlands

Fibrates are widely used hypolipidemic drugs which activate nuclear peroxisome proliferator-activated receptors (PPARs) and thereby affect the expression of different genes involved in lipid metabolism. Treatment with these drugs causes adverse changes in the biliary lipid composition and decreases the excretion of bile acids leading to an increased incidence of cholesterol gallstones. We studied the mechanism of regulation of bile acid synthesis and cholesterol 7α-hydroxylase (7αOH) and sterol 27-hydroxylase (27OH) gene expression by fibrates in cultured hepatocytes and in vivo in rats and mice. Ciprofibrate (300µM) and the PPARα agonist Wy14,643 (100µM) decreased bile acid synthesis in rat heptocytes by 66% and 61%, respectively. Ciprofibrate and Wy14,643 suppressed activities of 7αOH (-69% and -60%, respectively) and 27OH (both -49%), paralleled by a similar reduction of the respective mRNAs. In contrast, a high affinity ligand for PPARγ, BRL49653 had no effect. Treatment of rats with 0.05% (w/w) ciprofibrate decreased 7αOH enzyme activity (-87%) and mRNA levels (-69%). Evidence for the functional involvement of PPARα in the suppression of 7αOH and 27OH in vivo was obtained using PPARα null (-/-) mice. In wild-type mice, ciprofibrate reduced 7αOH and 27OH mRNA levels by 65% and 48%, respectively. However, in PPARα-/- mice this effect was completely abolished. Promoter-reporter studies showed the presence of a functional PPAR-responsive element in the proximal promoter of the 7αOH gene and/or interference with HNF-4-mediated activation of 7αOH gene, which both can mediate suppression by fibrates. A decreased production of bile acids by PPARα-mediated down-regulation of 7αOH may contribute to the increased risk for gallstone formation in patients treated with fibrates.