Z Ernährungswiss 36: 368–371 (1997)

*J. Scheibner
K. Lange
K. Empen
E. F. Stange

The contribution of newly synthesized cholesterol to biliary cholesterol in healthy humans

Die Bedeutung von neu synthetisiertem Cholesterin für die biliäre Cholesterinsekretion des gesunden Menschen

Summary Hypersecretion of biliary cholesterol appears to be the key defect in the pathogenesis of cholesterol gallstones, and this may be due to an enhanced synthesis of cholesterol. To measure fractional syntheses of biliary and plasma cholesterol, five male and 3 female healthy humans with an intact enterohepatic circulation were infused intravenously with [1-^{13}C]acetate for 15 h. Samples of duodenal bile and blood were taken hourly and an enteral formula diet was given. Free cholesterol mass distribution was analyzed by gas chromatography mass spectrometry. The Mass Isotopomer Distribution Analysis (MIDA) technique allowed to calculate fractional synthesis. After 6 hours of infusion, the [^{13}C]label of the cytosolic acetate pool reached a plateau of approximately 12 %. Individual fractional cholesterol synthesis in plasma and bile correlated significantly (6 – 15 h) and amounted to 4.2 % and 5.3 % after 15 h, respectively. It may be concluded from this study, that newly synthesized cholesterol is secreted into bile to a higher extent than into plasma.

Zusammenfassung Die Hypersekretion von biliärem Cholesterin scheint der Schlüsseldefekt in der Pathogenese der Cholesteringallensteine zu sein und ist möglicherweise bedingt durch eine erhöhte Cholesterinsynthese. Um die fraktionelle Synthese von biliärem Cholesterin und Plasmacholesterin zu messen, wurden 5 männliche und 3 weibliche gesunde Probanden mit einer intakten enterohepatischen Zirkulation intravenös mit [1-^{13}C]Acetat für 15 h infundiert. Proben duodenaler Galle und Blutproben wurden stündlich gewonnen und eine Formuladiät enteral verabreicht. Die Massenverteilung des freien Cholesterins wurde mittels Gaschromatographie mit Massenspektrometrie analysiert. Die Anwendung der Mass Isotopomer Distribution Analysis – (MIDA) – Technik erlaubte die Berechnung der fraktionellen Synthese. Nach 6stündiger Infusion erreichte die [^{13}C]Markierung des cytosolischen Acetatpools etwa 12 %. Die individuellen fraktionellen Cholesterinsynthesen im Plasma und in der Galle korrelierten signifikant miteinander (6 – 15 h) und betrugen 4,2 und 5,3 % nach 15 h. Aus dieser Studie wurde die Schlußfolgerung gezogen, daß neu synthetisiertes Cholesterin gegenüber dem Plasma zu einem höheren Anteil in die Galle sezerniert wird.

Key words [^{13}C]acetate – Mass Isotopomer Distribution Analysis – cholesterol synthesis – bile – human subjects

Schlüsselwörter [^{13}C]Acetat – Mass Isotopomer Distribution Analysis – Cholesterinsynthese – Galle – Mensch

*J. Scheibner · K. Lange · K. Empen
E. F. Stange
Department of Internal Medicine I
Division of Gastroenterology
Medical University of Lübeck
Ratzeburger Allee 160
D-23562 Lübeck
Germany

Introduction

Cholesterol hypersecretion into bile is the pathophysiological key defect that results in cholesterol gallstone formation. The putative hepatic mechanisms resulting in cholesterol hypersecretion are a decreased bile acid synthesis, a reduced cholesterol esterification, an increased lipoprotein uptake, or an increased cholesterol synthesis. Although the origin of biliary cholesterol is well-defined in experimental animals (1-3), only few data are available for healthy humans with an intact enterohepatic circulation (4). Employing [^{14}C]cholesterol to label preexisting cholesterol, Mitchell et al. (4) estimated, that only 5 % of biliary cholesterol was newly synthesized. Because inhibition of HMG-CoA-reductase, the key enzyme of cholesterol synthesis, reduces the cholesterol saturation of bile (5, 6), cholesterogenesis may play an important role for biliary cholesterol secretion. On the other hand, in obese humans and in most studies with cholesterol gallstone patients, an enhanced cholesterol synthesis could be demonstrated (7, 8) while an increased biliary cholesterol saturation is known for these individuals (9, 10). The specific aim of the present study was to directly quantitate the fractional synthesis of newly synthesized cholesterol secreted into bile and plasma of healthy volunteers employing the Mass Isotopomer Distribution Analysis (11, 12). In addition to the fractional synthesis, the absolute cholesterol synthesis of the whole body should be estimated in plasma and correlated to fractional synthesis of biliary cholesterol.

Study design and methods

Five male and 3 female healthy volunteers (20-28 years of age) with a normal body mass index, a good gallbladder contractility, and without gallstones were included in the study. To label newly synthesized cholesterol they received an intravenous infusion of 0.125 mmol [1-^{13}C] sodium acetate per kg body weight per h from 10 am to 1 am. In addition, adjusted to the individual's energy requirements a liquid formula was infused continuously via a nasoduodenal tubing with 3 lumina. During this period 1 ml of duodenal bile and 5 ml of plasma were collected hourly. These samples served for the measurement of fractional cholesterol syntheses. On day two to four, 9, 15, 21, 33, 39, 45, and 57 h after the end of [^{13}C]acetate infusion, the turnover rate of free plasma cholesterol was determined from multiple [^{13}C]labeled cholesterol.

Following its extraction by isopropanol – methanol/hexane and acetone/ethanol (13), unesterified biliary and plasma cholesterol was silylated by bis-(trimethylsilyl)-trifluoroacetamide, respectively. The cholesterol isotopomers with a mass to charge ratio of 368 (M_{+0}), 369 (M_{+1}), 370 (M_{+2}), 371 (M_{+3}), and 372 (M_{+4}) were quantitated by gas chromatography mass spectrometry and analyzed by Mass Isotopomer Distribution Analysis (MIDA) (11, 12). This technique included the measurement of the ratios of molar excesses of the individual cholesterol isotopomers from collected samples, but also the theoretical calculation of those ratios as a mathematical function of [^{13}C]acetate enrichment. Comparing measured and calculated ratios, especially of M_{+1} and M_{+3}, the cytosolic [^{13}C]acetate enrichments were estimated hourly. In this regard it is noteworthy that Hellerstein et al. (14, 15) demonstrated a good agreement of [^{13}C]acetate enrichments derived by this algorithm and directly measured enrichments. After the actual [^{13}C]acetate enrichments were derived as described above, the fractional syntheses of cholesterol in plasma and bile could be determined. The absolute whole body cholesterol synthesis rates during [^{13}C]acetate infusion were estimated in unesterified plasma cholesterol, which represents a fraction of the rapid exchangeable M1 pool. Therefore, a non-steady-state equation (12) was used, including the measured fractional synthesis, an assumed equal pool size of 9 g per person (16), and the measured turnover rate constant from M_{+3} of free plasma cholesterol.

Results and discussion

The percentage of isotopomer M_{+0} decreased from 73.9 % in native plasma cholesterol to 69.72 % following 15 h of [^{13}C]acetate infusion, whereas M_{+1}, M_{+2}, M_{+3}, and M_{+4}

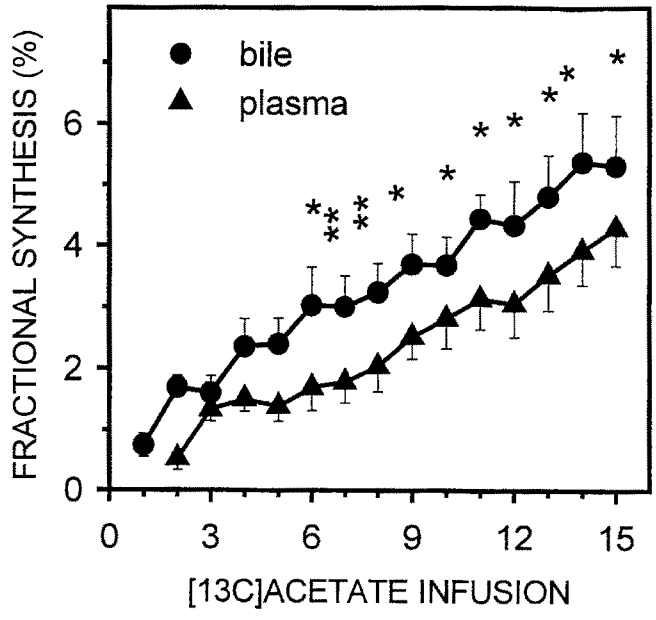

Fig. 1 Fractional synthesis of biliary and plasma cholesterol (in % of total cholesterol) during 15 h of [1-^{13}C]acetate infusion. Data are given as means ± 1 SEM of 8 healthy volunteers. * $p < 0.05$, ** $p < 0.01$ between bile and plasma (13).

increased from 22.97 to 23.33 %, from 3.50 to 4.75 %, from 0.37 to 1.25 %, and from 0.14 to 0.56 %, respectively. The mathematically predicted isotopomer distribution correlated well with that measured at the start of the infusion.

The [^{13}C]acetate enrichments estimated from biliary and plasma cholesterol showed an identical time course. Six hours after [^{13}C]acetate infusion they reached a plateau at approximately 12 %. We, therefore, speculate that de novo cholesterol secreted into bile and plasma may derive from a single acetate precursor pool (e.g., liver).

The fractional cholesterol synthesis in plasma (Fig. 1) increased continuously during [^{13}C]acetate infusion reaching 4.2 % after 15 h. Other investigators published a comparable fractional synthesis in plasma ranging from 3.8 % to 4.5 % using the MIDA technique (12,17). In addition, a fractional synthesis of 5 – 8 % resulted from the alternative deuterated water technique (18-20) after a longer experimental period of 24 h compared to 15 h in the present study.

From 6 to 15 h fractional synthesis of biliary cholesterol exceeded that of plasma cholesterol significantly by 19 – 44 % to reach 5.3 % at the end of [^{13}C]acetate infusion (Fig. 1). If each individual's data point from bile was plotted against that from plasma (Fig. 2), both fractional syntheses were highly correlated ($r = 0.868$; $p < 0.0001$). This relationship could also be shown for fractional synthesis in bile and total body cholesterol synthesis after 15 h ($r = 0.909$; $p < 0.01$; $Y_{[in\%]} = 0.055 + (1.22 * X)$). As a consequence, an increased whole body cholesterol synthesis, as measured in unesterified plasma cholesterol, is followed by an enhanced proportion of newly synthesized cholesterol in bile of healthy humans. Possibly, some newly synthesized cholesterol is directly secreted into bile without traversing the plasma compartment. At least, the pathophysiological relevance of this observation for biliary cholesterol hypersecretion remains unclear until the quantitative influence of an enhanced cholesterol synthesis on total biliary cholesterol secretion is demonstrated.

Regarding the measured turnover rate of 0.0143 ± 0.0019 from M_{+3}, a nearly constant total body cholesterol synthesis of 8.6 ± 1.6 mg/kg/d (602 mg/70 kg/d) could be estimated hourly after 6 h of [^{13}C]acetate infusion. Because cholesterol synthesis peaks at 6 am in human subjects (20), the constancy of the measured values argues for the validity of MIDA. Furthermore, this is in good agreement with studies employed alternative techniques, e.g., radioactive isotopic kinetic or sterol balance technique (21).

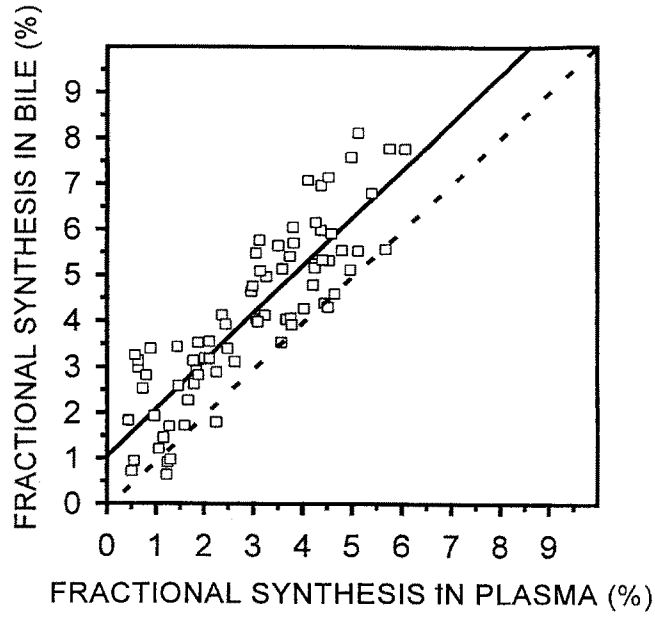

Fig. 2 Correlation between fractional synthesis measured in bile and plasma. Each data point represents one individual's synthesis measured hourly from hour 6 to 15 of the experiment (13).

Conclusions

Based on our data we conclude that newly synthesized cholesterol is preferentially secreted into bile in healthy humans. Future studies will investigate the effect of varying cholesterol synthesis rates on biliary secretion of newly synthesized and total cholesterol. This should allow a clarification of the pathophysiological relevance of an increased cholesterol synthesis for biliary cholesterol hypersecretion.

References

1. Scheibner J, Fuchs M, Hörmann E, Tauber G, Stange EF (1994) Biliary cholesterol secretion and bile acid formation in the hamster: The role of newly synthesized cholesterol. J Lipid Res 35:690-697
2. Scheibner J, Fuchs M, Schiemann M, Tauber G, Hörmann E, Stange EF (1993) Bile acid synthesis from newly synthesized vs. preformed cholesterol precursor pools in the rat. Hepatology 17:1095-1102
3. Turley SD, Dietschy JM (1981) The contribution of newly synthesized cholesterol to biliary cholesterol in the rat. J Biol Chem 256:2438-2446
4. Mitchell JC, Stone BG, Logan GM, Duane WC (1991) Role of cholesterol synthesis in regulation of bile acid synthesis and biliary cholesterol secretion in humans. J Lipid Res 32:1143-1149
5. Duane WC, Hunninghake DB, Freeman ML, Pooler PA, Schlasner LA, Gebhard RL (1988) Simvastatin, a competitive inhibitor of HMG-CoA reductase, lowers cholesterol saturation index of gallbladder bile. Hepatology 8:1147-1150
6. Mitchell JC, Logan GM, Stone BG, Duane WC (1991) Effects of lovastatin on biliary lipid secretion and bile acid metabolism in humans. J Lipid Res 32:71-78
7. Kern Jr F, (1994) Effects of dietary cholesterol on cholesterol and bile acid homeostasis in patients with cholesterol gallstones. J Clin Invest 93:1186-1194
8. Salen G, Nicolau G, Shefer S, Mosbach EH (1975) Hepatic cholesterol metabolism in patients with gallstones. Gastroenterology 69:676-684
9. Reuben A, Qureshi Y, Murphy GM, Dowling RH (1985) Effect of obesity and weight reduction on biliary cholesterol saturation and the response to chenodeoxycholic acid. Eur J Clin Invest 16:133-142
10. Bennion LJ, Grundy SM (1975) Effects of obesity and caloric intake on biliary lipid metabolism in man. J Clin Invest 56:996-1011
11. Hellerstein MK, Neese RA (1992) Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers. Am J Physiol 263:E988-E1001
12. Neese RA, Faix D, Kletke C, Wu K, Wang AC, Shackleton CHL, Hellerstein MK (1993) Measurement of endogenous synthesis of plasma cholesterol in rats and humans using MIDA. Am J Physiol 264:E136-E147
13. Empen K, Lange K, Stange EF, Scheibner J (1997) Newly synthesized cholesterol in human bile and plasma: quantitation by Mass Isotopomer Distribution Analysis. Am J Physiol 272:G367-G373
14. Hellerstein MK, Wu K, Kaempfer S, Kletke C, Shackleton CHL (1991) Sampling the lipogenic hepatic acetyl-CoA pool in vivo in the rat. Comparison of xenobiotic probe to values predicted from isotopomeric distribution in circulating lipids and measurement of lipogenesis and acetyl-CoA dilution. J Biol Chem 266:10912-10919
15. Hellerstein MK, Kletke C, Kaempfer S, Wu K, Shackleton CHL (1991) Use of mass isotopomer distributions in secreted lipids to sample lipogenic acetyl-CoA pool in vivo in humans. Am J Physiol 261:E479-E486
16. Gould RG, LeRoy GV, Okita GT, Kabara JJ, Keegan P, Bergenstal DM (1955) The use of C14-labeled acetate to study cholesterol metabolism in man. J Lab Clin Med 46:372-384
17. Faix D, Neese R, Kletke C, Wolden S, Cesar D, Coutlangus C, Shackleton CHL, Hellerstein MK (1993) Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis. J Lipid Res 34:2063-2075
18. Jones PJH, Dendy SM, Frohlich JJ, Leitch CA, Schoeller DA (1992) Cholesterol and triglyceride fatty acid synthesis in apolipoprotein E2-associated hyperlipidemia. Arteriosclerosis and Thrombosis 12:106-111
19. Jones PJH, Scanu AM, Schoeller DA (1988) Plasma cholesterol synthesis using deuterated water in humans: effects of short-term food restriction. J Lab Clin Med 111:627-633
20. Jones PJH, Schoeller DA (1990) Evidence for diurnal periodicity in human cholesterol synthesis. J Lipid Res 31:667-673
21. Grundy SM, Ahrens Jr EH (1969) Measurement of cholesterol turnover, synthesis and absorption in man, carried out by isotope kinetic and sterol balance methods. J Lipid Res 10:91-107