Low-fat, high-carbohydrate and high-fat, low-carbohydrate diets decrease primary bile acid synthesis in humans\textsuperscript{1–3}

Peter H Bisschop, Robert HJ Bandsma, Frans Stellaard, Anke ter Harmsel, Alfred J Meijer, Hans P Sauerwein, Folkert Kuipers, and Johannes A Romijn

ABSTRACT

Background: Dietary fat content influences bile salt metabolism, but quantitative data from controlled studies in humans are scarce.

Objective: The objective of the study was to establish the effect of dietary fat content on the metabolism of primary bile salts.

Design: The effects of eucaloric extremely low-fat (0%), intermediate-fat (41%; control diet), and extremely high-fat (83%) diets on kinetic values of cholate and chenodeoxycholate metabolism were determined after 11 d by using stable isotope dilution in 6 healthy men. All diets contained identical amounts of cholesterol.

Results: The total primary bile salt pool size was not significantly affected by dietary fat content, although the chenodeoxycholate pool was significantly higher during the low-fat diet. Fractional turnover rates of both primary bile salts were 30–50% lower during the low- and high-fat diets than during the control diet. Total hepatic bile salt synthesis was 30% lower during both the high- and low-fat diets, but synthesis rates of the 2 primary bile salts were differentially affected. The molar ratio of cholate to total bile salt synthesis increased from 0.50 ± 0.05 (SD) to 0.59 ± 0.05 and 0.66 ± 0.04 with increasing fat intake, whereas the molar ratio of chenodeoxycholate to total bile salt synthesis decreased from 0.50 ± 0.05 to 0.41 ± 0.05 and 0.34 ± 0.04. The relative concentration of deoxycholate in plasma increased during the low-fat period, which indicated increased absorption from the colon.

Conclusions: Both low- and high-fat diets reduce the synthesis and turnover rates of primary bile salts in humans, although probably through different mechanisms, and they affect the removal of cholesterol from the body. Am J Clin Nutr 2004;79:570–6.

KEY WORDS Diet, dietary fat, dietary carbohydrate, bile salt, cholate, chenodeoxycholate, deoxycholate, stable isotope

INTRODUCTION

The primary bile salts cholate and chenodeoxycholate are synthesized in the liver from cholesterol and, after conjugation to either taurine or glycine, are actively secreted into the bile. Bile salts are of key importance in the generation of bile flow and the control of bile salt pool size. Bile salt synthesis significantly contributes to the maintenance of whole-body cholesterol homeostasis (2). It is interesting that healthy humans show a wide variation in their rates of bile salt synthesis. In a series of normolipidemic subjects studied by Stellaard et al (3), the daily conversion of cholesterol to bile salts varied between 240 and 870 mg/d. Similar variations have been found in other studies (4–7). The reason for this variation is not known, but it may be related to diet composition, genetic factors, or both (8). Insight into the molecular regulation of the enterohepatic circulation of bile salts, as maintained by the combined actions of hepatic and intestinal transporter systems and the bile salt synthetic machinery in the liver, has greatly increased in the past few years (9, 10). Transcription factors have been identified that control the expression of bile salt transporters and of key enzymes in bile salt synthesis in response to alterations in bile salt flux and cellular cholesterol content (11). Translation of this knowledge to human (pathologic) conditions will be a major challenge in the coming years.

Dietary factors may affect bile salt kinetics through several mechanisms. Several older observations (12–14) indicate that the rate of gallbladder emptying in response to a meal contributes to regulation of bile salt pool size in humans, mainly through effects on the fractional turnover rate (FTT) of bile salts. Studies by Duane and Hanson (13), Duane (15), and Veysey et al (16) showed that short bowel transit time is another factor involved in the control of bile salt pool size. Dietary fat content and composition have been shown to influence the regulation of bile salt synthesis have been shown to influence the regulation of bile salt synthesis.

\footnotesize{\textsuperscript{1} From the Departments of Endocrinology & Metabolism (PHB and HPS) and of Biochemistry (AJM), Academic Medical Center, University of Amsterdam; the Center for Liver, Digestive and Metabolic Diseases, Department of Pediatrics, University Hospital Groningen, Groningen, Netherlands (RHJB, FS, AhH, and FK); and the Department of Endocrinology, Leiden University Medical Center, Leiden, Netherlands (JAR).
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\textsuperscript{3} Reprints not available. Address correspondence to PH Bisschop, Department of Endocrinology and Metabolism (F5), Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, Netherlands. E-mail: p.h.bisschop@amc.uva.nl.
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synthesis in experimental models. Cheema et al (17) reported that, in mice, the response of Cyp7a, the gene encoding cholesterol 7α-hydroxylase, to dietary cholesterol is dependent on the type of dietary fat. In rats, a fat-free diet reduced bile salt synthesis rates (18), and, conversely, fat feeding stimulated bile salt synthesis (19). Relatively few studies have addressed the effects of dietary fat content and composition on human bile salt kinetics. Lindstedt et al (20) found no consistent effects on cholate pool size and turnover in healthy subjects fed solid diets or fat-rich liquid formula diets containing either corn oil or coconut oil. Hepner (14) reduced gallbladder contraction in humans by institution of a fat- and protein-restricted diet and found increased cholate and chenodeoxycholate pool sizes, decreased FTRs, and no significant effects on synthesis rates. Finally, Andersen and Hellström (21) noted small but significant increases in cholate and chenodeoxycholate synthesis rates without changes in their pool sizes in patients with hyperlipoproteinemia after they switched from a diet in which 60% of the energy was supplied as fat to a diet in which 60% of the energy was supplied as carbohydrate. However, these diets were prepared from natural foods, and thus the daily cholesterol intake during high-fat feeding was almost twice that during high-carbohydrate feeding. So far, there are no reports of controlled studies in which the effects of low- and high-fat diets with fixed cholesterol contents and those of an intermediate-fat (control) diet on human bile salt metabolism were quantified and compared. The specific aim of this study was therefore to establish the effects of large variations in the amounts of dietary fat with a fixed cholesterol content on kinetic values of bile salt metabolism in healthy volunteers. Eucaloric diets with extreme differences in dietary fat intake (ranging from 0% to 83% of total energy) were used to maximize short-term effects of dietary fat content on bile salt metabolism.

SUBJECTS AND METHODS

Subjects

Six healthy men [aged 29–55 y; body mass index (BMI; in kg/m²): 21–26] were studied on 3 separate occasions. All subjects were in good health and were not using any medication. All participating subjects gave written informed consent. This study was approved by the Medical Ethical Committee of the Academic Medical Center, Amsterdam.

Diets

Each subject was studied on 3 different diets. The diets were eucaloric and consisted of a liquid formula (tailor-made by Nutricia, Zoetermeer, Netherlands) that contained identical amounts (15% of total energy) of protein. In addition to the proteins, the low-fat diet contained only carbohydrates (85% of total energy), the intermediate-fat (control) diet contained both carbohydrate and fat (44% and 41% of total energy, respectively), and the high-fat diet contained mainly fat and some carbohydrate (83% and 2% of total energy, respectively). Each diet provided 300 mg cholesterol/d. The ratio of saturated:monounsaturated:polyunsaturated fatty acids was 2:2:1 for all diets containing fat. Detailed composition of the diets is shown in Table 1. The sequence of the 3 studies was determined by random assignment. Energy requirements for each subject were assessed by a dietitian by means of a 3-d dietary journal. Meals with predetermined amounts of calories were taken at 6 fixed time points between 0800 and 2130 each day for 11 d. In addition to the diets, the subjects were allowed to drink only water ad libitum. The subjects were free-living during the study. All subjects refrained from alcohol, and exercise was limited to normal daily activities during the experimental diets. All diets were well tolerated by the subjects. The subjects consumed their habitual diet for 8–10 wk in the periods between the experimental diets.

Protocol

The experimental diets were consumed for a period of 11 d. At 1800 on day 8 of each experimental diet, a blood sample was taken 1 h after ingestion of a standardized amount of the liquid diet for background measurements. Subsequently, 50 mg [2,2,4,4-2H₄]cholate and 50 mg [2,2,4,4-2H₄]chenodeoxycholate (Isotec Inc, Miamisburg, OH) dissolved in 200 mL of 0.5% NaHCO₃ were administered orally. On days 9, 10, and 11 of the experimental periods, blood samples were taken at 0900 and 1800, each time 1 h after ingestion of a standardized amount of the liquid diet, for determination of [2,2,4,4-2H₄]cholic acid and [2,2,4,4-2H₄]chenodeoxycholic acid plasma enrichments. Plasma was separated by centrifugation (Hettich Rotanta/ RP; Dext BV, de Bilt, Netherlands) at 3000 rpm for 10 min at 4 °C and stored at −80 °C until it was analyzed.

Sample preparation

Bile salts were derivatized according to Stellaard et al (4). Briefly, 50 µL plasma was taken and bile salts were hydrolyzed overnight at 37 °C with the use of 30 µL cholyglycine hydrolyse in an acetate buffer (pH 5.6). After extraction of free bile, salt samples were treated with 100 µL of 10% pentafluorobenzyl bromide (PFB) solution in acetonitrile for 20 min at room temperature. The PFB esters were extracted, and dried samples were treated with 100 µL bis-trimethylsilyl-trifluoroacetamide-
pyridine-trimethylchlorosilane (5:4:1; by vol). Samples were kept in this solution for analysis.

Gas chromatography

Plasma concentrations of the major individual bile salt species were quantified by gas chromatographic procedures as described previously (22) and used to calculate total plasma bile salt concentrations in postprandial samples where indicated.

Gas chromatography–mass spectrometry

All gas chromatography–mass spectrometry analyses were performed on a Finnigan SSQ7000 Quadrupole GC-MS instrument (Finnigan MAT, San Jose, CA). Gas chromatography separation was performed on a 30 m × 0.25 mm column with a 0.25-µm film thickness (DB-5MS; J&W Scientific, Folsom, CA). We injected 1–3 µL of the final solution of derivatized bile salts in the splitless mode at a injector temperature of 290 °C and a column temperature of 150 °C. The temperature was then programmed to remain at 150 °C for 1 min and then rise at a rate of 30 °C/min to 315 °C, where it remained for 14 min. The ion source was operated in the negative ion chemical ionization mode at 150 °C, and methane was applied as the moderating gas. Isotope ratios were determined at mass-to-charge ratios of 623.3 (M0) and 627.3 (M4) for cholate and of 535 (M0) and 539 (M4) for chenodeoxycholate.

Plasma cholesterol and triacylglycerol concentrations

Plasma triacylglycerol concentrations were measured on a centrifugal analyzer (COBAS BIO; Roche Diagnostics Boehringer Mannheim, Mannheim, Germany) with the use of another enzymatic method (Triacylglycerol GPO-PAP; Roche Diagnostics Boehringer Mannheim). Total cholesterol concentration was measured on a Hitachi 747 analyzer (Roche Diagnostics Boehringer Mannheim, Mannheim, Germany) with the use of another enzymatic method [CHOD-PAP (1489704); Roche Diagnostics Boehringer Mannheim].

Calculations and statistics

Kinetic values were calculated according to Stellaard et al (3, 4). From the decay of the enrichment of the isotope ratio M4:M0 over time, expressed as the natural logarithm, the FTR and the pool size are calculated with the use of the formula

\[
\text{Pool size} = (D \times b \times 100/e^a) - D
\]

where \( D \) is the administered amount of label, \( b \) is the isotopic purity, and \( a \) is the intercept with the \( Y \) axis of the enrichment versus time curve. The rate of synthesis is then calculated by multiplying pool size and FTR.

Data were compared by analysis of variance for randomized block design, which was followed by the Bonferroni test when appropriate. A \( P \) value < 0.05 was considered significant. Data are presented as means ± SDs.

RESULTS

Total plasma cholesterol concentrations were 3.58 ± 1.10, 3.91 ± 0.76, and 3.47 ± 0.59 mmol/L after the low-fat, control, and high-fat diets, respectively (NS). As expected, the fasting plasma triacylglycerol concentration was higher after the low-fat diet than after either the control or the high-fat diet (1.90 ± 1.47, 0.83 ± 0.47, and 0.79 ± 0.27 mmol/L, respectively; \( P < 0.05 \)). Postprandial plasma bile salt concentrations determined at 1 h after the ingestion of a fixed amount of the diet were significantly lower during the control and low-fat diets (13.3 ± 0.7 and 13.1 ± 1.5, respectively) than during the high-fat diet (18.4 ± 2.2 µmol/L, \( P = 0.003 \)), which suggested a stronger gallbladder contraction during the high-fat diet.

The decline in enrichment of \([2,2,4,4-2H_2]\)cholate and \([2,2,4,4-3H_2]\)chenodeoxycholate in plasma was linear during all diets. Representative data for one person during the 3 diets are shown in Figure 1. The mean values for pool size, FTR, and synthesis rate of all subjects during the 3 diets are summarized in Table 2.

Pool size

The pool size of cholate was not affected by dietary fat content. Although the chenodeoxycholate pool was significantly larger during the low-fat diet than during the other diets (\( P < 0.016 \)), the combined pool size of the 2 primary bile salts did not differ significantly between the 3 diets (\( P = 0.33 \)).

Fractional turnover rate

The FTR of the cholate pool was lower during both the low-fat (−28%; \( P = 0.112 \)) and high-fat (−42%; \( P = 0.042 \)) diets than...
during the control diet. The FTR of the chenodeoxycholate pool was significantly lower during the low-fat (−53%; P = 0.009) and high-fat (−45%; P = 0.023) diets than during the control diet.

**Synthesis**

The synthesis of cholate was clearly lower during both the low- and high-fat diets (−33%; P = 0.012 and −16%; P = 0.120, respectively) than during the control diet. The synthesis of chenodeoxycholate was significantly lower during the high-fat diet only (−42%; P = 0.001). When combined, total bile salt synthesis was 23% lower (P = 0.033) during the low-fat diet and 28% lower (P = 0.011) during the high-fat diet than during the control diet. As a consequence, the total amount of cholesterol converted to bile salt was significantly less during the low-fat (447 ± 136 mg/d) and high-fat (417 ± 145 mg/d) diets than during the control diet (614 ± 170 mg/d).

Dietary fat content differentially affected synthesis rates of both primary bile salts (Figure 2). With increasing fat intake, the molar ratio cholate synthesis:total bile salt synthesis increased from 0.50 ± 0.05 to 0.59 ± 0.05 to 0.66 ± 0.04, whereas the molar ratio chenodeoxycholate synthesis:total bile salt synthesis decreased from 0.50 ± 0.05 to 0.41 ± 0.05 to 0.34 ± 0.04.

To evaluate whether the change in bile salt synthesis was reflected in bile salt pool composition, the relative contribution of the major bile salt species was measured in postprandial plasma. There was a tendency to an increased contribution of the secondary bile salt deoxycholate during both the low- and the high-fat diets, whereas the relative cholate content increased with increasing fat content (Figure 3). As a result, the deoxycholate:cholate in plasma, which reflected the input of the secondary bile salt into the circulating bile salt pool, increased during the low-fat diet only, to 2.54 ± 0.70; the ratio during the control and high-fat diets was 0.58 ± 0.17 and 0.87 ± 0.27, respectively.

**DISCUSSION**

In the present study we aimed to establish the effects of alterations in dietary fat content on human bile salt metabolism. In a randomized, crossover study, healthy persons were studied during the consumption of a diet with an extremely low fat content, a diet with an intermediate fat content that resembled their habitual Western-type diet, and a diet with an extremely high fat content. The use of liquid formula diets allowed for accurate substitution of carbohydrates for fat without changing fatty acid and carbohydrate composition or cholesterol intake. Accordingly, the effects observed in the individual subjects can be attributed to variations in the ingested amounts of carbohydrate and fat only. We found that both low-fat and high-fat diets rapidly reduce the FTRs of the circulating pools of cholate and chenodeoxycholate. Synthesis of cholate is lower during the consumption of a low-fat diet, whereas ingestion of an extremely high-fat diet is associated with lower synthesis rates of both primary bile salts. Consequently, the conversion of cholesterol to bile salts is markedly affected by dietary fat content.

Extrapolation of the results of the present study to actual consumed diets should be done with care, because we cannot exclude the possibility that the addition of only a small amount of fat to the extremely low-fat diet would have altered bile salt kinetics. In addition, it should be realized that the kinetic values derived from the approach used are strongly interrelated, as illustrated by the inverse relation between FTR and pool size in humans reported by Watkins et al (23). Therefore, the actual metabolic sequence of events underlying the observed effects of dietary fat content on human bile salt metabolism cannot be deduced. Yet the data do allow us to translate some of the novel insights into the regulation of bile salt metabolism to human physiology.

In view of the studies summarized in the Introduction (12–14), it is highly likely that impaired turnover rates of primary bile salts during low-fat feeding in our study are attributable, at least in part, to delayed gallbladder emptying. The increased contribution of the secondary species deoxycholate to the pool at the expense of cholate may reflect higher spillover of cholate to the colon (ie, less effective ileal reabsorption), bacterial translocation, or both during this feeding regimen. Impairment of gastrointestinal motility, such as that due to the secretion of less cholecystokinin, may contribute: it has been shown that slow intestinal transit is associated with increased input of deoxycholate into the total bile salt pool (24). It is surprising that similarly reduced turnover rates of cholate and chenodeoxycholate were found after the consumption of the extremely high-fat diet. Under these conditions, gallbladder contraction presumably is maximally stimulated, and it can be anticipated that the
cycling frequency of the bile salt pool is higher than that during the low-fat diet. The high postprandial bile salt concentrations found in high-fat–fed subjects support the notion that the inges-
tion of such a diet induces a stronger gallbladder contraction. If this is the case, impaired FTRs can be achieved only by more efficient reabsorption from the intestine. Whether that requires up-regulation of intestinal bile salt uptake systems is not known. Intestinal bile salt reabsorption is mediated by the apical sodium–dependent bile salt transporter localized to the terminal ileum (1). It has been reported that an increased intestinal bile salt load induces the expression and the transport capacity of this transport system in rodents (25), but opposite results have also been published (26–28). Furthermore, expression of the ileal bile acid–binding protein, an intestine-specific cytosolic protein putatively involved in the absorption process (29, 30), is dramatically increased by bile salts in rodent ileum, ileal organ explants, and cultured Caco-2 cells via activation of the nuclear farnesoid X receptor or bile salt receptor (31, 32). A farnesoid X receptor response element has been identified in the promoter of the human ileal bile acid–binding protein gene (33). Thus, it is tempting to speculate that a high dietary fat content induces an enhanced capacity for bile salt reabsorption from the intestine in humans and, consequently, a reduced fecal loss or, in kinetic terms, a reduced turnover of the circulating bile salt pool. Despite the reduced turnover rates induced by both low- and high-fat feeding regimens, the total pool size of primary bile salts did not change, although the chenodeoxycholate pool was somewhat larger during the low-fat diet. Less synthesis of bile salts during the low-fat diet than during the control diet may be explained by the relatively greater contribution, during the low-fat diet, of the secondary bile salt deoxycholate to the (slowly) circulating pool. Animal studies by Schreibner et al (34) indicated that this relatively hydrophobic dihydroxy bile salt is very effective in suppressing gene expression and activity of cholesterol 7α-hydroxylase (CYP7A), but whether that applies also to the human situation is not known. The suppressive effect of bile salts on CYP7A expression is also mediated by the farnesoid X receptor (11). The suppression of bile salt synthesis during the high-fat diet, on the other hand, may be a consequence of farnesoid X receptor activation by enhanced flux of bile salt through the liver due to an accelerated cycling frequency, which, in turn, may be due to the stimulation of gallbladder emptying and induced expression of the bile salt reabsorption machinery in the distal ileum. In addition, fatty acids per se may influence hepatic bile salt synthesis. Control of genes involved in hepatic fatty acid metabolism is orchestrated, to a major extent, by peroxisome proliferator–activated receptor (PPAR) α. PPARα is a ligand-activated transcription factor that belongs to a subfamily of the nuclear receptor gene family (35) and that is activated by fatty acids and their derivatives. Several studies (36–39) indicated a role for PPARα in the control of expression of both CYP7A and CYP27; the latter is the gene that encodes 27-hydroxylase and catalyzes the first step of the so-called neutral pathway of bile.
salt biosynthesis. The fat-dependent increase in the cholate:chenodeoxycholate synthesis ratio may also reflect a PPARα-mediated phenomenon. The ratio at which both primary bile salts are synthesized is determined by the activity of sterol 12-hydroxylase (9, 10). Hunt et al (40) established that the expression of the murine gene encoding sterol 12-hydroxylase (CYP8B) is controlled by PPARα. Treatment of wild-type mice with the PPARα-agonist WY14,643 leads to the induction of CYP8B mRNA in the liver and to a greater proportion of cholate in bile. These effects were abolished in PPARα-knockout mice. The human CYP8B gene has been cloned (41), but no data are yet available on its regulation. Our data indicate that the activity of the enzyme encoded by this gene is induced by dietary fat.

It should be realized that, in addition to the direct nutritional effects of the extreme diets used in this study, hormonal influences may have contributed to the final outcome. For instance, glucocorticoids decrease cholesterol 7α-hydroxylase activity and expression (42, 43), whereas thyroid hormone exerts the opposite effect (44, 45). In the present study, glucocorticoid concentrations were not affected by diet composition, but T4 was markedly decreased during the high-fat diet (data not shown), which might have contributed to the decrease in bile salt synthesis. In contrast, low plasma insulin concentrations during the high-fat diet (46) would be expected to have an opposite effect, because insulin has a suppressive effect on bile salt synthesis (47).

We conclude that both low-fat and high-fat diets have major effects on human bile salt metabolism, which are reflected in decreased turnover and synthesis of primary bile salts and, consequently, in impaired removal of cholesterol from the body via bile salts. We postulate that different mechanisms underlie these similar metabolic effects of the low-fat and high-fat diets.

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