Fish Oil Increases Bile Acid Synthesis in Male Patients with Hypertriglyceridemia

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ABSTRACT Fibrates are drugs of choice in patients with hypertriglyceridemia (HTG), but may increase the risk for gallstones by decreasing bile acid synthesis. Fish oil might be a therapeutic alternative, but its effect on bile acid metabolism in humans is unknown. We compared the effects of triglyceride-lowering therapy by fish oil or bezafibrate on cholesterol synthesis and bile acid metabolism in HTG. Cholesterol synthesis, bile acid pool sizes, and synthesis rates were compared between 9 male HTG patients and 10 normolipidemic controls matched for age, sex, and BMI. Effects of bezafibrate or fish oil were studied only in HTG patients in a randomized crossover trial. Patients had 14-fold higher serum triglyceride concentrations and greater cholesterol synthesis, as indicated by a 107% higher ratio of serum lathosterol to cholesterol (P < 0.01) than controls. The groups did not differ in bile acid metabolism. Both bezafibrate and fish oil reduced serum TG concentration (−68 and −51% vs. baseline, respectively). Compared with baseline, bezafibrate therapy was associated with reduced cholesterol synthesis (−25%, P = 0.009) without changes in bile acid synthesis rate and pool size. In contrast, fish oil increased bile acid synthesis (+31% vs. baseline, P = 0.07 and +53% vs. bezafibrate, P = 0.02) and altered bile acid distribution, as reflected by an increased ratio of the cholic acid (CA) synthesis rate to the chenodeoxycholic acid (CDCA) synthesis rate (+35% vs baseline, P = 0.05 and +32% vs bezafibrate, P = 0.07) without effects on bile acid pool size or cholesterol synthesis. In conclusion, cholesterol synthesis is greater in HTG patients than in controls, whereas bile acid synthesis does not differ. Bezafibrate and fish oil have similar triglyceride-lowering capacities, but distinct effects on cholesterol synthesis. Bile acid synthesis is increased by fish oil, but not by bezafibrate therapy.


KEY WORDS: • triglycerides • bezafibrate • fish oil • bile acid synthesis • hypertriglyceridemia

Hypertriglyceridemia (HTG) is associated with cardiovascular disease (1), pancreatitis (2) and cholesterol gallstone formation (3,4). The disorder is characterised by elevated plasma TG concentrations, mainly in very-low-density-lipoprotein (VLDL), low levels of HDL-cholesterol, and insulin resistance. Several studies have addressed cholesterol (5–8), and bile acid metabolism (6,9–14) in HTG, mainly in relation to the increased risk for gallstone disease. In general, these studies revealed the presence of bile supersaturated with cholesterol (12,15) as well as increased cholesterol and bile acid synthesis in HTG (3,7–12).

Fibrates are commonly used in the treatment of HTG patients in order to prevent cardiovascular disease by lowering TG levels (16). However, fibrates may increase the risk for cholelithiasis (17–19) by increasing biliary cholesterol saturation (20) through increased biliary cholesterol secretion and inhibition of bile acid synthesis (21,22). Fish oil, that also effectively lowers plasma TG levels (23), has been shown to have profound favorable effects on cardiovascular risk (24) and is a therapeutic alternative for fibrates in HTG patients (25). However, the effects of fish oil on cholesterol synthesis and bile acid metabolism in humans are unknown. Therefore the aim of this study was to evaluate the effects of hypertriglyceridemia (HTG) on cholesterol synthesis and bile acid metabolism as well as to compare triglyceride-lowering therapy by bezafibrate and fish oil on cholesterol synthesis and bile acid metabolism in patients with HTG.

**SUBJECTS AND METHODS

Subjects and study design. This study is part of a larger study investigating the effects of HTG and lipid lowering therapy on biliary lipid secretion and gallbladder motility. The subjects and study design as well as specific analytical techniques have been published in detail.
(25). In short, the study population consisted of 9 unrelated male patients with endogenous HTG and 10 normal lipidemic, age-, sex- and BMI-matched healthy control subjects. The diagnosis endogenous HTG was based on the mean of two fasting blood samples obtained after a step 1 diet of the NCEP (26) for at least 8 wk. The presence of asymptomatic gallstones or sludge was excluded by ultrasound. If the patients used lipid-lowering therapy before the study-onset, this was stopped for at least 6 wk prior to the study entry. None of the participants took any medication known to affect cholesterol or bile acid metabolism.

The study started with the assessment of baseline values in both controls and HTG patients: fasting blood samples were taken for determination of lathosterol and cholesterol concentrations at visit 1. At visit 2 (one wk later) fasting blood samples were taken for the assessment of serum lipids and bile acids, after which measurement of bile acid kinetics was started as detailed below. For this purpose, during 5 consecutive days postprandial morning and evening blood samples were taken. Hereafter, HTG patients were randomized to receive in a cross-over fashion either bezafibrate (Bezalip retard®, Hoffmann-La Roche Ltd, Basel, Switzerland), 400 mg once daily or fish oil 5 g/day (Triglyceride-lowering Lube, Hadsund, Denmark) containing 3.6 g (n-3) fatty acids (eicosapentaenoic acid, C20:5(n-3); 1.9 g and docosahexaenoic acid C22:6(n-3) 1.1 g)) for 7 wk. These two treatment periods were separated by a 6-wk washout period without lipid-lowering medication. At the onset and in week 7 of the treatment periods fasting blood samples were obtained. Serum lipids and cholesterol synthesis were investigated in week 6, whereas in week 7 of each treatment period bile acids synthesis was determined. Informed consent was obtained from each participant and the protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center.

**Sample collection and lipid analysis.** Blood sampling and serum lipid, glucose, insulin and total bile acid analyses were performed as described previously (25,27). Insulin resistance was assessed using the homeostasis model approximation (HOMA) by the following formula: insulin resistance = insulin/(22.5e(1-1glucone)).

**Cholesterol synthesis.** The serum lathosterol to cholesterol ratio was used as indicator of cholesterol synthesis (28). Total (free and esterified) serum cholesterol was measured colorimetrically, whereas total lathosterol was assayed by gas-liquid chromatography (28).

**Bile acid synthesis and pool sizes.** Participants had a standardized breakfast (3263 kJ, 50 g fat, 42 g protein, 30 g carbohydrates) at the research laboratory at 0830 AM. At 1030 AM a 6-ml blood sample was taken to determine the isotope distribution of endogenous bile acids. Thereafter, 50 mg [2,2,4,4,1-He] Cholic Acid (CA) and 50 mg [2,2,4,4,2-He] Chenodeoxycholic Acid (CDCA) (CDN Isotopes, Ponte-Claire, Quebec, Canada) dissolved in 200 mL 0.5% NaHCO3 were taken. On the next 5 d blood samples were taken 2 h after both breakfast and dinner.

Deuterium enrichments for both CA and CDCA were measured simultaneously in 50 μL plasma samples by gas chromatography mass spectrometry (GC/MS), selected ion-monitoring (29) applying negative ion chemical ionization (NICI). Some modifications were necessary to overcome problems due to the high triglyceride concentration in plasma of the patients. This affected the stability in recovery, chromatographic peak shape and retention time as well as the stability in mass spectrometry performance. Therefore, the protocol was modified as follows. The sample obtained after enzymatic deconjugation (29) of 200 μL plasma was extracted twice with 4 mL hexane to remove neutral lipids. Thereafter the residual sample was acidified and extracted with diethylether to continue the original protocol. The gas chromatography separation was slightly modified using a 2 m × 0.25 mm (0.25μm film thickness) OV1701 column (CPSil 19CB, Chrompack BV, Middelburg, The Netherlands) as a guard column between the injector and the 15 m × 0.25 mm (0.25μm film thickness) DB5ms (J&W Scientific, Folsom, CA, U.S.A.) analytical column as described. These modifications improved the long-time stability of the measurements. The selected ion monitoring parameters were extended for the measurement of isotope enrichment for chenodeoxycholic acid PFB TMS derivative: m/z 535 and 539.

**Calculations.** From the isotope enrichment the pool size, the fractional turnover rate and the synthesis rate were calculated for CA and CDCA (30). From the calculated CA and CDCA pool sizes and the fraction of deoxycotic acid (DCA) in the biliary bile acid profile, the DCA pool size was determined. The total bile acid synthesis was calculated as the sum of the CA synthesis rate and CDCA synthesis rate.

**Data and statistical analysis.** Results are expressed as means ± SEM. Differences between the HTG patients and controls were tested using the Mann-Whitney-U-test. Differences between HTG patients at baseline and after bezafibrate or fish oil therapy were evaluated by ANOVA and the post-hoc LSD test. Linear regression analysis was performed to identify whether insulin, glucose or insulin resistance as group-associated confounders contributed to the results. P-values ≤ 0.05 were considered significant.

**RESULTS**

**Baseline characteristics.** Age, sex, BMI and fasting serum glucose levels did not differ between HTG patients and controls. In contrast, fasting insulin levels (+35%, P = 0.059) as well as the HOMA-index as a measure of insulin resistance (+45%, P = 0.034) were higher in HTG patients than in controls. By definition, HTG patients had higher serum total TG and VLDL-TG concentrations than controls, whereas the other lipoprotein fractions also differed from those in controls (Table 1).

**Effects of fish oil and bezafibrate on serum biochemistry in HTG patients.** Serum lipid levels in HTG patients did not differ between the first “baseline” determination and measurements performed before the start of the bezafibrate and fish oil treatment periods. Therefore, only serum lipid levels at the end of these periods were compared (Table 1). Both fish oil and bezafibrate decreased total serum TG, VLDL-TG, total cholesterol and VLDL cholesterol concentrations, whereas low-density-lipoprotein (LDL)- and HDL-cholesterol concentrations increased (Table 1). Lipid-lowering effects did not differ between fish oil and bezafibrate, except for the VLDL-TG concentration, which was 35% lower after bezafibrate than after fish oil therapy (P = 0.04).

Bezafibrate therapy did not affect fasting glucose, fasting insulin levels and the degree of insulin resistance (Table 1). In contrast, fish oil significantly increased all three compared to baseline (+7%, P = 0.023; +36%, P = 0.007 and +44%, P = 0.01, respectively) and to bezafibrate therapy (+7%, P = 0.02, +39%, P = 0.006 and +49%, P = 0.007, respectively, Table 1).

**Cholesterol synthesis.** Cholesterol synthesis was higher in HTG patients than in age-, sex- and BMI-matched controls as deduced from the 107% higher ratio of serum lathosterol to cholesterol (Fig. 1, P = 0.008). Fish oil did not affect cholesterol synthesis in HTG patients, whereas bezafibrate therapy decreased this ratio compared to baseline (−25%, P = 0.009) as well as to after fish oil therapy (−22%, P = 0.024, Fig. 1). Regression analysis showed that serum glucose and insulin concentrations and insulin resistance did not contribute to the increased cholesterol synthesis in HTG patients (all P > 0.23).

**Bile acid synthesis and pool sizes.** The biliary bile acid compositions (CA, CDCA and DCA) were similar in HTG patients and were not affected by fish oil or bezafibrate therapy (data not shown). Bile acid synthesis rates, fractional turnover rates and pool sizes did not differ between HTG patients and controls (Table 2). Fish oil tended to increase CA- and total bile acid synthesis rates (+45%, P = 0.071 and +31%, P = 0.079, respectively) as well as CA fractional turnover rate (+20%, without effects on CDCA synthesis rate, and fractional turnover rate, or individual and total pool sizes. As a consequence, the ratio of the CA synthesis rate to the CDCA synthesis rate was 35% greater after fish oil therapy compared to baseline (P = 0.05, Table 2). Bezafibrate therapy did not affect...
CA-, CDCA-, DCA- and total bile acid pool sizes, and CA-, CDCA-, and total bile acid synthesis rates, or fractional turnover rates in HTG patients.

In comparison to bezafibrate therapy, fish oil resulted in higher CA (+68%, $P = 0.02$), CDCA (+28%, $P = 0.05$), and total bile acid synthesis rates (+53%, $P = 0.02$) and a 32% greater ratio of the CA synthesis rate to the CDCA synthesis rate ($P = 0.07$, Table 2).

**DISCUSSION**

This study shows that HTG patients have increased cholesterol synthesis, whereas their bile acid metabolism did not differ from age-, sex- and BMI-matched controls. Furthermore, we observed that fish oil and bezafibrate therapy exerted similar lipid-lowering effects in HTG patients, but had distinctly different effects on cholesterol and bile acid metabolism. Fish oil increased bile acid synthesis, specifically CA synthesis, without effects on bile acid pool size or cholesterol synthesis. Bezafibrate, in contrast, significantly decreased cholesterol synthesis, while it tended to decrease bile acid synthesis as well.

In line with earlier studies (5–8), HTG patients showed an increased cholesterol synthesis in comparison to controls even after correction for BMI (intrinsically by BMI-matching) and insulin resistance (by regression analysis). At first sight these data conflict the study of Duane et al. demonstrating that cholesterol synthesis did not differ from BMI-matched controls in comparison with patients with FHTG (31). However, it should be noted that this study focused on FHTG, a genetically distinct entity of HTG. Furthermore, one outlier in the control group of that study markedly influenced the results, and exclusion of this outlier revealed a tendency to increased cholesterol synthesis in FHTG as well.

Bile acid synthesis, fractional turnover rates and bile acid pool sizes did not significantly differ in HTG patients compared to those of controls, disputing earlier studies, demonstrating increased bile acid FTR (10,12) and increased bile acid synthesis (6,9–12,14) in HTG patients. However, in these studies there were large inter-individual differences with regard to abnormalities in bile acid metabolism (14). Moreover, in those studies HTG patients had higher BMIs than controls and obesity itself is associated with increased bile acid synthesis (32,33), whereas weight reduction is associated with reduced bile acid synthesis in HTG (34). The lack of significant differences in our study might be explained by the fact that HTG patients were compared to BMI-matched controls. In addition, it could be that we did not observe differences in bile acid metabolism in HTG patients due to the small-sample size of our study population. However, we assume that large differences would have been noted in our study.

Earlier studies showed reductions in both cholesterol- and bile acid synthesis upon fibrate therapy in both humans (21,22) and mice (35). These effects may be explained by fibrates-induced PPARα-mediated down-regulation of cholesterol 7α-hydroxylase and sterol 27-hydroxylase gene expression (22), whereas fibrates have been shown to diminish cholesterol biosynthesis through inhibition of HMG-CoA synthase as well.

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls Baseline</th>
<th>Bezafibrate Baseline</th>
<th>Fish oil Baseline</th>
<th>Controls Bezafibrate</th>
<th>Bezafibrate Bezafibrate</th>
<th>Fish oil Bezafibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.9 ± 2.3</td>
<td>52.6 ± 2.7</td>
<td>28.0 ± 0.7</td>
<td>28.2 ± 0.7</td>
<td>10.6 ± 0.5</td>
<td>10.6 ± 0.5</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>27.2 ± 0.9</td>
<td>27.8 ± 0.6</td>
<td>28.0 ± 0.7</td>
<td>28.2 ± 0.7</td>
<td>10.6 ± 0.5</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>86.4 ± 3.6</td>
<td>89.2 ± 2.5</td>
<td>90.5 ± 2.9</td>
<td>90.0 ± 2.7</td>
<td>100.5 ± 9.3</td>
<td>100.5 ± 9.2</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.5 ± 0.1</td>
<td>5.9 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>6.2 ± 0.2</td>
<td>3.7 ± 0.4</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Insulin resistance, HOMA-index</td>
<td>2.5 ± 0.3</td>
<td>3.7 ± 0.4*</td>
<td>3.6 ± 0.5</td>
<td>5.3 ± 0.9*</td>
<td>3.7 ± 0.4*</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Total Cholesterol, mmol/L</td>
<td>5.20 ± 0.32</td>
<td>6.32 ± 1.31*</td>
<td>6.03 ± 0.54</td>
<td>6.95 ± 1.16</td>
<td>6.03 ± 0.54*</td>
<td>6.95 ± 1.16</td>
</tr>
<tr>
<td>Total Triglycerides, mmol/L</td>
<td>1.09 ± 0.21</td>
<td>15.56 ± 3.34</td>
<td>4.90 ± 1.31†</td>
<td>7.58 ± 2.85†</td>
<td>3.51 ± 1.09†</td>
<td>3.51 ± 1.09†</td>
</tr>
<tr>
<td>VLDL-Cholesterol, mmol/L</td>
<td>0.29 ± 0.09</td>
<td>5.86 ± 1.28*</td>
<td>2.22 ± 0.60</td>
<td>3.51 ± 1.09†</td>
<td>3.51 ± 1.09†</td>
<td>3.51 ± 1.09†</td>
</tr>
<tr>
<td>LDL-Cholesterol, mmol/L</td>
<td>0.72 ± 0.18</td>
<td>13.80 ± 3.19*</td>
<td>3.86 ± 1.22†</td>
<td>5.97 ± 2.09†</td>
<td>3.86 ± 1.22†</td>
<td>5.97 ± 2.09†</td>
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<tr>
<td>HDL-Cholesterol, mmol/L</td>
<td>3.98 ± 0.31</td>
<td>1.74 ± 0.22†</td>
<td>3.12 ± 0.29†</td>
<td>2.71 ± 0.26†</td>
<td>3.12 ± 0.29†</td>
<td>2.71 ± 0.26†</td>
</tr>
</tbody>
</table>

† Values are means ± SEM, n = 9 (HTG patients) or 10 (controls).
* Different from controls, $P \leq 0.05$.
† Different from HTG patients at baseline, $P \leq 0.05$.
‡ Different from HTG patients after bezafibrate treatment, $P \leq 0.05$.
as HMG CoA reductase (36). In our study we did not observe a decrease in bile acid synthesis during bezafibrate therapy, which may be due to the small sample size of the study.

The hypotriglyceridemic effect of fish oil is well known (23) and confirmed in our study. In contrast, the effect of fish oil on insulin-resistance is less clear: some studies observed no effect (37), whereas others, similar to our observation documented an increase in insulin resistance upon fish oil (38).

This is the first study describing the effects of fish oil on bile acid synthesis in humans, whereas only scarce data are available in animals. In rats, fish oil has been shown to increase cholesterol secretion into bile (39,40), and to increase bile acid pool size and synthesis rate (41), although others found no effect on bile acid synthesis in rats (39) or hamsters (42). Furthermore, fish oil increased cholesterol 7α-hydroxylase gene expression in mice (43). These differences in bile acid metabolism may be due to the animal model used (44). In the present study fish oil increased bile acid synthesis, specifically CA synthesis in comparison to both baseline and bezafibrate. This observation is of interest, since over the past years several groups suggested a reciprocal relationship between TG synthesis and bile acid synthesis: upon treatment with bile acid-binding resins serum TG concentration increased (45,46) whereas upon treatment with CDCA TG levels decreased (47,48). To the best of our knowledge this is the first study describing divergent effects on serum triglyceride levels and bile acid synthesis, suggesting that the coupling between bile acid synthesis and triglyceride synthesis may not be as direct as previously suggested and may involve alternative mechanisms. The mechanism behind the hypotriglyceridemic action of fish oil is unknown. Whereas the hypotriglyceridemic effect of fibrates is attributed to activation of PPARα, experiments with PPARα knock-out mice (49) showed that the triglyceride lowering effect of fish oil was not abolished, indicating that this action of fish oil is not mediated by PPARα. Recently, it was reported that fish oil may decrease hepatic VLDL production by stimulating apoB degradation via post-ER-pre-secretory proteolysis or PERPP (50). Further studies are required to elucidate the underlying mechanism of triglyceride-lowering in relation to bile acid synthesis upon fish oil treatment.

In conclusion, the current study demonstrates that in HTG patients cholesterol synthesis was increased compared to BMI matched controls, while bile acid pool size and synthesis were similar in both groups. In HTG patients however, bezafibrate significantly reduced cholesterol synthesis, without a significant effect on bile acid synthesis. Fish oil exerted similar TG-lowering capacities, increased bile acid synthesis, without affecting cholesterol synthesis.

LITERATURE CITED


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