Hypocholesterolemic effects of fatty acid bile acid conjugates (FABACs) in mice

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Abstract

Fatty acid bile acid conjugates (FABACs) prevent and dissolve cholesterol gallstones and prevent diet induced fatty liver, in mice. The present studies aimed to test their hypocholesterolemic effects in mice. Gallstone susceptible (C57L/J) mice, on high fat (HFD) or regular diet (RD), were treated with the conjugate of cholic acid with arachidic acid (FABAC; Aramchol). FABAC reduced the elevated plasma cholesterol levels induced by the HFD. In C57L/J mice, FABAC reduced plasma cholesterol by 50% (p < 0.001). In mice fed HFD, hepatic cholesterol synthesis was reduced, whereas CYP7A1 activity and expression were increased by FABAC. The ratio of fecal bile acids/neutral sterols was increased, as was the total fecal sterol excretion. In conclusion, FABACs markedly reduce elevated plasma cholesterol in mice by reducing the hepatic synthesis of cholesterol, in conjunction with an increase of its catabolism and excretion from the body.

Keywords: Cholesterol; Bile acid; Fatty acid; Liver; Lipoproteins; Neutral sterols; Gallstone; Mice

Fatty acid bile acid conjugates (FABACs)2 are synthetic lipid molecules recently designed to solubilize biliary cholesterol. FABACs were shown to delay, reduce, or prevent cholesterol crystallization in vitro and ex vivo in bile model solutions and in human bile [1]. FABACs have also been shown to prevent the formation of cholesterol gallstones and to dissolve preexisting gallstones in experimental animal models [2,3]. Several FABACs have been synthesized [1,2]. The FABACs containing the longer chain fatty acids, arachidic (C-20) and stearic (C-18), were shown to be preferable in relation to cholesterol metabolism. Accordingly, Aramchol (C-20 FABAC) was used in the present studies, in which the bile acid moiety is represented by cholic acid.

In addition to the effects on gallstones, FABACs have been found to have other metabolic effects. They prevented or reduced the formation of fatty liver, induced by high fat diets in mice, hamsters and rats [4]. Aramchol increased fecal sterol output in rats [5]. Recent studies have shown that FABACs induce cholesterol and phospholipid efflux from human fibroblasts in an ABCA1-dependent and Apo AI-independent manner [6]. It was also observed that in rodents on high fat diets, FABAC therapy reduced the elevated plasma cholesterol levels.

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The present studies were initiated to test and validate the hypocholesterolemic effects of Aramchol and to investigate its mechanism of action in gallstone susceptible C57L/J mice.

Materials and methods

Animals and diets

Inbred, gallstone susceptible C57L/J male mice, 4–5 weeks old, were purchased from the Jackson Laboratory (Maine, USA), and used in most experiments. Regular powdered rodent chow diet (4% fat, RD) was purchased from Koffolk (Petch Tikva, Israel). The high fat diet (HFD) was prepared as previously described [7] and consisted of 15% butter fat, 1% cholesterol, 0.5% cholic acid and 2% corn oil added (w/w) to RD.

Mice were kept on a 12 h day/night cycle and had free access to water and food. The studies were approved by the institutional committee for animal experiments at Tel-Aviv University, Tel-Aviv, Israel.

Experimental procedure

The FABAC used in the present experiments was 3β-arachidyl amido, 7α, 12α-dihydroxy, 5β-cholan, 24-oic acid, which is an arachidonic acid conjugate (Aramchol; C-20 FABAC). Animals were fed RD or HFD. Aramchol was given by gavage at a dose of 25 mg/kg/day or 150 mg/kg/day (FABAC 25 or FABAC 150, respectively) suspended in 0.1 ml saline. Control animals received saline by oral gavage. After the experimental period, all animals were given ketamine anesthesia, and sacrificed. Blood was drawn from the abdominal aorta and collected in tubes with 0.01% EDTA. Plasma was separated by centrifugation and kept frozen at −20 °C for further determinations. The gallbladder was ligated, excised and inspected. Bile was aspirated with a thin (23G) needle. Thereafter, the gallbladder was opened and inspected for the presence of gallstones and crystals, using a stereoscopic light microscope and polarized light. The liver was excised and immediately frozen in liquid nitrogen for further analyses.

Design of studies

Three sets of different experiments were performed. In the first set, the molecular effects of FABAC on plasma cholesterol were studied in C57L/J after four weeks of treatment. Animals were fed RD or HFD. FABAC was administered at a dose of 25 or 150 mg/kg/day. In the second set of experiments, the molecular effects on cholesterol and bile acid metabolism of FABAC were studied in C57L/J mice. In these experiments, animals were either on RD or on HFD. FABAC was administered at a dose of 25 or 150 mg/kg/day for a period of 2 weeks. In the last set of experiments, animals were treated for a period of time ranging between 3 and 8 weeks in order to evaluate the effects of FABAC on plasma cholesterol levels.

Bile analyses

Biliary lipids were extracted by chloroform:methanol (2:1, v:v) and phase separation, according to Folch et al. [8]. Bile salts and cholesterol were quantitated enzymatically [9,10]. Phospholipids were determined by the method of Bartlett [11]. Because of minute volumes, bile was pooled and statistical significance could not be tested. Cholesterol saturation index (CSI) was calculated according to Carey’s critical table [12].

Preparation of liver microsomes, enzyme assay, and cholesterol determination

Liver biopsies were homogenized with a loose-fitting Teflon pestle in nine volumes of 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose, 10 mM DTT, 10 mM EDTA, and 50 mM NaCl. The homogenate was then centrifuged at 20,000g for 15 min at 4 °C. The supernatant was thereafter centrifuged at 100,000g for 60 min. Half of the microsomal fraction was resuspended in the homogenizing medium and spun at 100,000g for 60 min. The 100,000g pellet was suspended in 20 mM imidazole buffer, pH 7.4, containing 10 mM DTT in a volume corresponding to that of the 20,000g supernatant, and used for the assay of the 3-hydroxyl-3-methylgluthathyl CoA reductase (HMGCoAR). The other half of the original microsomal fraction was recenterfuged at 100,000g in the absence of DTT. The pellet was suspended in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA, and used for assay of cholesterol 7α-hydroxylase (CYP7A1) activity, determined as the formation of 7α-hydroxycholesterol (pmoles/mg protein/min) from endogenous microsomal cholesterol using isotope dilution-mass spectrometry [13]. Microsomal HMGCoAR activity was assayed by determining the conversion of HMG CoA to mevalonate, and expressed as pmoles formed per mg protein per min [14]. The enzyme assays were carried out in duplicate. The concentrations of free and total cholesterol in the liver homogenates and the microsomal fractions were determined by isotope dilution-mass spectrometry after addition of deuterium-labeled cholesterol as an internal standard, as previously described [15,16]. Esterified cholesterol levels were calculated as the difference between the total and free cholesterol.

Chemical analysis of plasma

In the first set of experiments, an Advia 1650 autoanalyzer (Bayer, Japan) was used for cholesterol determinations. In the second set of experiments, total cholesterol and triglycerides were determined in pooled plasma using a Monarch automated analyzer (ILS Laboratories Scandinavia AB, Sollentuna, Sweden). The kit for the determination of triglycerides detects free glycerol, serum triglyceride (TG) values were corrected with regard to their respective glycerol content. Size-fractionation of lipoproteins and their cholesterol and triglyceride content was performed on 10 μl of pooled plasma from each group by size exclusion chromatography (SEC) using a 300 x 3.2 mm Superox 6B column (Amerham Pharmacia Biotech AB, Uppsala, Sweden) at a flow rate of 40 μl/min as described in detail by Parini et al. [17].

Unesterified lathosterol was determined by isotope dilution-mass spectrometry after the addition of a deuterium-labeled cholesterol as an internal standard [18].

Total nucleic acid (TNA) preparation and analysis of mRNA levels

Frozen liver specimens (0.4 g) from each animal were homogenized in 4 ml of SET buffer [1% (w/v) sodium dodecyl sulphate, 10 mmol/L EDTA, and 20 mmol/L Tris–HCl, pH 7.5] with a Polytron (Kinematica, type PT 10/35, Kriens, Lucerne, Switzerland). The samples were subsequently sonicated on ice by two 5 s pulses, in a Branson B 15 Sonifier, and digested with proteasome K (200 μg/ml) for 45 min at 45 °C. TNA was precipitated with ethanol after phenol–chloroform extraction, and the pellet was suspended in 300 μl of 20% SET buffer. LDL-receptor mRNA, CYP7A mRNA, and HMGCoAR mRNA were quantitated with a solution hybridization titration assay, as previously described [19]. The slopes of the linear hybridization signals were calculated by the method of least squares, and compared with the slope generated by synthetic mRNA standards. Data are expressed as Arbitrary Units, calculated from moles mRNA/μg TNA.

Analysis of fecal sterols

Feces were collected during the last 72 h of the experimental period. Fecal lipids were analyzed by GLC according to Batta et al. [20]. In brief, 75 mg of lyophilized feces were butylated by addition of 1 ml n-butyl alcohol and 0.1 ml 6 N HCL. Nor-cholic acid and 5α-cholastane were added as internal standards. The mixture was incubated for 4 h at 60 °C and thereafter the solvents evaporated at 60 °C under air stream. Samples were silylated with 1 ml Sil-Prep kit (Alttech, Alttech II, USA) at 55 °C for 30 min and thereafter resuspended in 1 ml hexane, centrifuged, and
supernatant were transferred to new vials and brought to a final volume of 50 μl. A sample of 1 μl was applied to an SGE 30 m × 0.25 mm × 0.25 μm capillary GLC column. The results were analyzed with a Clarity program (Data Apex Ltd., Czech Republic).

Statistics

Data were analyzed by two-tailed Student’s t-test or by Mann–Whitney test. P < 0.05 was considered statistically significant. Results are presented as mean ± SD. When appropriate, data were analyzed by 1-way ANOVA, followed by post-hoc comparisons according to LSD test (Statistica software, Stat Soft, Tulsa OK). Data shows mean ± SEM, if not otherwise indicated.

Results

Effects of FABAC and dietary challenge on body weight, liver weight, gallstone formation and bile composition

At first, we wanted to evaluate the effects of diet and FABAC treatment on body weight, liver weight, gallstone formation, and bile composition in gallstone susceptible C57L/J mice. Administration of FABAC did not influence food intakes. Approximately 5 g/day/animal were consumed by mice receiving either FABAC or saline, independent of the treatment and of the duration of the experiments. Animals fed HFD had a tendency to consume less food (4.7 g/day/animal). No significant changes in body weights were observed in the animals after either 4 weeks or 2 weeks of treatment (Table 1). Challenge for 4 weeks with HFD had a tendency to increase liver weight (from 0.6 ± 0.1 g in RD group to 1.0 ± 0.3 g in HFD group; NS). Treatment with FABAC did not further affect liver weight in these animals.

After sacrifice, gallbladders were opened and cholesterol crystals were observed in 70% of the animals fed HFD for 2 weeks. In the animals fed HFD for 4 weeks, cholesterol gallstones were observed in 100% of the gallbladders. Treatment with FABAC resulted in the disappearance of both crystals and/or gallstones.

After 2 weeks of treatment, FABAC did not appreciably change biliary cholesterol concentration in the animals on RD. An increase in the phospholipid/(phospholipid + bile salts) ratio was however observed from 0.19 to 0.23 and 0.28 at low and high FABAC dose, respectively). FABAC 25 and FABAC 150 also decreased the cholesterol saturation index (CSI) from 1.98 to 1.29 and 1.48, respectively.

In gallstone susceptible C57L/J mice, challenge for 2 weeks with HFD resulted in a rise of total biliary lipids from 4.66 g% (RD) to 7.79 g% (HFD). FABAC had a tendency to decrease biliary phospholipid and to increase bile salt concentration in bile (NS), at both doses. As bile samples were pooled—due to small volumes—the statistical significance of the changes in biliary lipids could not be tested.

Plasma lipid concentration and lipoprotein profiles

Total plasma triglycerides were not affected by FABAC therapy in animals fed RD. (Fig. 1A, left). Animals on HFD started from higher triglyceride levels. In these animals, FABAC lowered triglycerides in a dose dependent manner to a level comparable to that of mice on RD (Fig. 1A, right).

In mice on RD, FABAC did not affect plasma cholesterol (Fig. 1B, left). Similarly, also in mice on HFD, FABAC did not affect plasma cholesterol to a marked degree, in these short term experiments (Fig. 1B, right). Unfortunately in this latter experiment plasma samples from mice on the HFD were pooled, the standard deviations and significance could not be calculated.

In mice fed RD, there were only minor effects of FABAC on triglyceride lipoprotein profiles. HFD markedly raised plasma triglycerides for the most part in the VLDL and LDL fractions (Fig. 2A, right). FABAC, at both doses, markedly decreased plasma triglycerides, particularly in the VLDL (Fig. 2A, right). As expected separation of lipoproteins by SEC in plasma from RD fed animals demonstrated that most cholesterol was carried in the HDL fraction. FABAC moderately reduced HDL cholesterol in a dose dependent manner (Fig. 2B, left).

Table 1

<table>
<thead>
<tr>
<th>Exp./diet</th>
<th>Exp./length (weeks)</th>
<th>FABAC dose (mg/kg BW/day)</th>
<th>Animals (n)</th>
<th>Crystals (%)</th>
<th>Gallstones (%)</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
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<tr>
<td>RD 4</td>
<td>—</td>
<td></td>
<td>12</td>
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<td>0</td>
<td>18.8 ± 1.5</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>HFD 4</td>
<td>—</td>
<td></td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>22.1 ± 1.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>21.5 ± 0.9</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>21.9 ± 0.8</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>RD 2</td>
<td>—</td>
<td></td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>22.8 ± 1.5</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>23.9 ± 1.0</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td></td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>21.4 ± 1.5</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>HFD 2</td>
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<td></td>
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<td>0</td>
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<td>0.9 ± 0.2</td>
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<tr>
<td></td>
<td>150</td>
<td></td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>21.6 ± 1.8</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

RD, regular chow diet; HFD, lithogenic diet.
while simultaneously decreasing HDL and slightly VLDL cholesterol.

**Hepatic cholesterol composition**

Table 2 shows the effect of diet and FABAC on hepatic total and microsomal cholesterol concentrations in gall-stone susceptible C57L/J mice. On RD, FABAC administration did not significantly influence total hepatic and microsomal cholesterol. However, a rise in the esterified cholesteryl fraction was observed (Table 2). After 4 weeks HFD, hepatic total cholesterol concentration rose markedly to 55 ± 7.8 nmol/mg protein. FABAC treatment did not significantly change total, free or esterified cholesterol at both doses. Microsomal total cholesterol concentration rose markedly to 55 ± 7.8 nmol/mg protein. FABAC treatment did not significantly change total, free or esterified cholesterol at both doses. Microsomal total cholesterol concentration rose significantly from 95 ± 4.8 to 114 ± 5.7 nmol/mg protein on FABAC 150. The change was related to the significant increase of the cholesteryl ester concentration from 21 ± 3.9 in the HFD mice to 36 ± 0.9 nmol/mg protein on FABAC 150 (p < 0.01).

**Fecal sterols and bile acids**

Analysis of the total fecal sterol output—neutral and acidic sterols, and plant sterols—showed a marked increase from 16 ± 8.7 to 107 ± 36 μmol/day/100 g BW after HFD. Total fecal sterol output further increased by 50% to 157 ± 2 μmol/day/100 g BW when FABAC 150 was given (Fig. 3A). The increase following the addition of FABAC was mainly due to a significant increase in fecal bile acids from 13 ± 10 to 81 ± 20 (μmol/day/100 g BW, p < 0.05; Fig. 3B). The amounts of phytosterols (μmol/day/100 g BW) were 5.6 ± 4.5 in controls on RD, 12 ± 4.4 on HFD, and 13 ± 4.2 on HFD treated with FABAC 150, and thus not affected by FABAC and in line with the similar food intake among the different groups of animals.

**Enzymes regulating hepatic cholesterol metabolism**

In animals fed RD, FABAC 25 increased CYP7A activity significantly, from 12 ± 7.4 to 21 ± 4.3 (p < 0.05; Fig. 4A). No effects were seen with FABAC 150. CYP7A mRNA decreased progressively with FABAC administration in the RD mice—significance could not be tested due to pooled samples. In mice fed HFD for 4 weeks (Fig. 4B), the presence of cholic acid in the HFD reduced CYP7A activity. Treatment with FABAC completely abolished the negative feed-back of cholic acid on CYP7A activity and induced a significant increase of circa 2- and 3-fold by low- and high-dose, respectively. The CYP7A mRNA rose over 2-fold with FABAC 150.
In animals on HFD, FABAC 150 for 2 weeks reduced HMGCoAR activity from 58 to 36 pmol/min/mg (p < 0.05; Fig. 5A). The lathosterol/cholesterol ratio, which also indirectly reflects cholesterol synthesis, was increased by the HFD after 4 weeks treatment (Fig. 5B). Treatment with FABAC 25 decreased the ratio by over 50% (p < 0.05), whereas FABAC 150 produced an unexplained rise in the lathosterol/cholesterol ratio (Fig. 5B).

The mRNA levels of HMGCoAR also rose on the HFD, as compared to mice on RD. FABAC therapy reduced the increase by 63% and 67% at the low and at the high dose respectively (p < 0.01 for both doses), so that
the resulting levels were below that of animals on RD. The mRNA levels of the LDL receptor followed an almost identical pattern (Fig. 5B). It increased from 8.4 to 10.7 on the HFD and decreased to 6.1 and 4.7 on FABAC 25 and FABAC 150, respectively (*p < 0.05 and **p < 0.001, respectively). In mice fed RD, FABAC caused no changes in LDLr mRNA levels after 2 weeks.

Effects of long-term treatment with FABAC on plasma total cholesterol in (gallstone susceptible) mice

In order to evaluate whether long-term treatment with FABAC affected total plasma cholesterol, gallstone susceptible C57L/J mice fed HFD were tested after different treatment periods (Fig. 6). FABAC 150 consistently reduced plasma total cholesterol by circa 50% after 4, 6, and 8 weeks of treatment, respectively (p < 0.05 and p < 0.001, respectively). In mice fed RD, FABAC caused no changes in LDLr mRNA levels after 2 weeks.

Discussion

The present study reveals several new and important findings regarding the effect of Aramchol, a C-20 fatty acid bile acid conjugate (FABAC), on hepatic lipid metabolism in general and on sterol metabolism in particular.

In gallstone susceptible C57L/J mice the FABAC caused major reductions in plasma cholesterol levels when animals were on a high cholesterol, bile acid, and saturated fat diet (HFD). A significant (−56%) decrease in plasma cholesterol has also been seen in non-gallstone susceptible C57Bl6/J mice (unpublished observation). The reduction in plasma cholesterol was maintained throughout the FABAC treatment period and seemed to be dose-dependent. No effects on total plasma cholesterol were on the contrary observed in mice on standard chow (RD), suggesting that FABACs may protect animals from high dietary lipid load.

The cholesterol lowering effect of FABAC seems to be the result of different potential mechanisms acting simultaneously on hepatic cholesterol metabolism. A reduction in hepatic cholesterol synthesis seems to follow FABAC treatment. This was confirmed by analysis of both the hepatic microsomal activity of HMGCoAR and by measurement of plasma lathosterol/cholesterol level. However when FABAC was given at 150 mg/kg/day, a discrepancy between the hepatic microsomal activity of HMGCoAR (reduced) and the plasma lathosterol/cholesterol levels (increased) was observed. If the hepatic microsomal activity of HMGCoAR reflects the capacity of the liver to synthesize cholesterol, the lathosterol/cholesterol ratio reflects the total body cholesterol synthesis. Thus, the discrepancy observed may reflect a possible induction of cholesterol synthesis in the extra-hepatic organs, which may compensate for the hepatic effects of the compound. FABAC seems also to be able to control the level of expression of HMGC-oAR gene leading to a clear decrease of the mRNA levels in the liver, an observation that further supports the hepatic effects of FABAC in reducing cholesterol synthesis. Also, FABAC showed major effects on the catabolism of cholesterol and on its excretion from the body. As expected, the cholic acid present in the HFD reduced the activity and decreased the gene expression of CYP7A1. At any dose, FABAC was remarkably able to blunt the negative feed-back of cholic acid on bile acid synthesis leading to an increase in the activity and expression of CYP7A1. A similar effect on CYP7A1 activity was also noted when FABAC was administered at low dose to animals on RD. Nevertheless in these animals, no stimulatory effects of FABAC were seen on the expression of the CYP7A1 gene. FABAC resulted in a dose dependent decrease in CYP7A1 mRNA levels in the RD fed animals. However the positive effects of FABAC on bile acid synthesis and CYP7A1 activity were further supported by the changes found in fecal sterol excretion. The cholesterol rich HFD markedly increased the excretion of fecal cholesterol and its metabolites (animal sterols). Concomitant FABAC therapy greatly increased the bile acid/cholesterol ratio in the feces, consistent with an increased synthesis of bile acids from cholesterol. Alternatively, FABAC may prevent the absorption of the dietary cholic acid resulting in increased fecal bile acid excretion and positively affecting bile acid synthesis in animals on HFD. This, however, is
an untested assumption. It should be noted that the chemical and GC methods used do not detect FABAC nor did we find evidence for FABAC deconjugation to bile acids. These effects in mice of FABAC were previously also observed in rats [5], in which Aramchol treatment increased fecal cholesterol and bile acid excretion. Interestingly, the increased synthesis of bile acids by FABAC did not result in increased biliary bile acid concentrations, possibly due to the observed increase in fecal bile acid excretion induced by FABAC. Recently, fibroblast growth factor 15 (FGF15) has been identified as a major intestinal mediator of the negative feed-back that bile acids exert on their own synthesis [21] and it would be interesting to understand whether FABAC affect the intestinal expression of FGF15. Unfortunately, all the experiments were performed prior to the report on FGF15 and intestinal mucosa was not collected preventing us from addressing this in the present report.

HFD induced gallstones in all C57L/J mice after 30 days and FABAC treatment completely prevented the gallstone formation independently of the dose, as reported before [2]. While gallstone prevention and dissolution by FABACs has been conclusively proven, the mechanism(s) of the effect remains uncertain. In C57L/J mice, HFD unexpectedly increased the activity and the mRNA of HMGCoAR, an aberrant metabolic response that has been previously reported in this mouse strain and attributed to defective regulation of SREBP2 [22]. As mentioned above, FABAC was more effective in mice on HFD than in mice on RD, suggesting that FABAC may enhance particularly those cholesterol homeostatic mechanisms activated by excess dietary cholesterol. Brown and Goldstein [23] described the events in the hepatocyte activated by cholesterol entering into the cell: HMGCoAR activity was decreased, cholesterol esterification was increased and LDL receptor synthesis was decreased. It is remarkable that FABACs induced all of these effects in addition to stimulating CYP7A activity and cholesterol efflux from cells and the intestine. Such a chain of coordinated effects raises the question of central regulation. At this point there is insufficient evidence to speculate whether nuclear receptors are involved and/or whether membrane sterol sensors are activated [24,25].

In the present study FABAC reduced plasma triglycerides, again only in animals on the HFD. Analysis of plasma lipoproteins showed that the effect of FABAC consisted in a decrease of triglycerides mainly in the VLDL fraction. These findings are relevant to the observation that FABACs were shown to reduce the hepatic lipid content during induction of fatty liver, in several animal models [4].
reduction in the hepatic lipid content was predominantly due to an effect on hepatic triglycerides. All together these observations suggest that FABACs do not promote an increased triglyceride excretion from the liver, and that the reduction in hepatic triglyceride content seems secondary to a decreased synthesis or increased catabolism or a combination of both. Nevertheless, it should be mentioned that FABACs have been shown to have variable effects on plasma triglycerides in several experimental animal model suggesting that species differences can modulate the effects of FABACs on triglyceride metabolism.

As expected, HFD induced increases in liver and microsomal total cholesterol which was accounted for by major increases in esterified cholesterol, mainly in the microsomal fraction. FABAC therapy at the high-dose further increased the content of esterified cholesterol mainly in the microsomes. This effect of FABACs should be closely monitored; preliminary studies on total ACAT activity in the liver gave conflicting results. Further studies addressing whether ACAT2 or ACAT1, or both, are stimulated by FABAC will be of fundamental importance.

In summary, the present study showed that FABACs have strong plasma cholesterol lowering effects in gallstone susceptible C57L/J mice. A decreased hepatic synthesis of cholesterol, secondary to a decrease in HMGCoAR activity, with a simultaneous increase in cholesterol catabolism to bile acids, via an increase in CYP7A1 activity, seem to explain the effects of FABAC in plasma. Those effects associated to increased fecal sterol losses may contribute to a reduction of total body cholesterol, and suggest a potential therapeutic role for these molecules.

Acknowledgments

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