Abolished synthesis of cholic acid reduces atherosclerotic development in apolipoprotein E knockout mice

Katharina Släti, Mats Gåfvels, Kristina Kannisto, Olga Ovchinnikova, Gabrielle Paulsson-Berne, Paolo Parini, Zhao-Yan Jiang, and Gösta Eggertsen

Unit for Clinical Chemistry, Department of Laboratory Medicine and Centre for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Abstract To investigate the effects of abolished cholic acid (CA) synthesis in the ApoE knockout mouse [apolipoprotein E (apoE) KO], a double-knockout (DKO) mouse model was created by crossbreeding Cyp8b1 knockout mice (Cyp8b1 KO), unable to synthesize the primary bile acid CA, with apoE KO mice. After 5 months of cholesterol feeding, the development of atherosclerotic plaques in the proximal aorta was 50% less in the DKO mice compared with the apoE KO mice. This effect was associated with reduced intestinal cholesterol absorption, decreased levels of apoB-containing lipoproteins in the plasma, enhanced bile acid synthesis, reduced hepatic cholesteryl esters, and decreased hepatic activity of ACAT2. The upregulation of Cyp7a1 in DKO mice seemed primarily caused by reduced expression of the intestinal peptide FGF15. Treatment of DKO mice with the farnesoid X receptor (FXR) agonist GW4064 did not alter the intestinal cholesterol absorption, suggesting that the action of CA in this process is confined mainly to the formation of intraluminal micelles and less to its ability to activate the nuclear receptor FXR. Inhibition of CA synthesis may offer a therapeutic strategy for the treatment of hyperlipidemic conditions that lead to atherosclerosis.—Släti, K., M. Gåfvels, K. Kannisto, O. Ovchinnikova, G. Paulsson-Berne, P. Parini, Z.-Y. Jiang, and G. Eggertsen. Abolished synthesis of cholic acid reduces atherosclerotic development in apolipoprotein E knockout mice. J. Lipid Res. 2010. 51: 3289–3298.

Supplementary key words atherosclerosis • bile acids • metabolism

The pathogenesis of atherosclerosis is a multifactorial process comprising a number of pathological conditions, such as dyslipidemia, lipid deposition in the arterial wall, inflammation, cell migration, and thrombosis formation (1). The role of bile acids in this disease process also deserves attention, inasmuch as bile acids tightly regulate cholesterol homeostasis. The hepatobiliary system constitutes a major clearance pathway for excess cholesterol, which is eliminated into the fecal route either through conversion of cholesterol into bile acids or by direct secretion of cholesterol in the biliary. Cholic acid (CA), one of the major primary bile acids in both man and rodents, is assigned a number of functions within this metabolic route. Intestinal absorption of cholesterol is promoted by CA, in which its role as a specific ligand for the nuclear farnesoid X receptor (FXR), regulates the expression of a large number of genes within the lipid metabolism and also the carbohydrate metabolism [for a review, see Meir and Leitersdorf (2)]. The contribution of the small intestine in the regulation of cholesterol metabolism and in the development of atherosclerosis is underlined by the observation that high dietary administration of cholesterol, with or without cholate supplementation, accelerates the vascular lesions in apolipoprotein E (ApoE) knockout mice (apoE KO) (3), a commonly used animal model for atherosclerosis [for a review, see Meir and Leitersdorf (4)].

The direct uptake of cholesterol in the small intestine is mainly mediated by NPC1L1 [for a review see Davis and Altman (5)]. Nevertheless, CA also plays a role in intestinal cholesterol absorption, because it promotes the uptake by inclusion of luminal cholesterol into micelles. In mice, abolished synthesis of CA by targeted disruption of the P-450 cytochrome Cyp8b1 reduces the intestinal cholesterol uptake by about 50% (6). Accordingly, CA-depleted mice also show other effects on cholesterol metabolism: they do not accumulate cholesteryl esters (CEs) in the...
liver when fed cholesterol-enriched diets (6), and have increased bile acid synthesis and bile acid pool sizes. We have previously shown that in mice, CA also seems to be the most-potent ligand for FXR (7).

To more carefully investigate the role of CA in a condition of disturbed cholesterol metabolism and atherosclerosis, we crossed the apoE KO with the Cyp8b1 knockout (Cyp8b1 KO) strain (8) to create an experimental mouse model that is prone to developing atherosclerosis but unable to synthesize CA. Our results show that apoE KO mice devoid of CA have only half the amount of atherosclerotic lesions and much lower levels of plasma cholesterol despite upregulated cholesterol synthesis. We also show that the action of CA in this process is most probably confined to formation of intraluminal micelles and, to a minor extent, its potency to activate the nuclear receptor FXR.

MATERIALS AND METHODS

Chemicals

Sodium cholate (>99%) and cholesterol (>99%) were purchased from Sigma-Aldrich, St. Louis, MO. The FXR agonist GW4064 was synthesized by Synthecol, Inc., Forskningsparken Ideon, Lund, Sweden, and was suspended in 1% methylcellulose before administration. [5,6-3H]β-sitostanol was obtained from American Radiolabel Chemicals, Inc., St. Louis, MO and [4-13C] cholesterol from Amersham Biosciences, Sweden.

Animals and experimental procedures

Mice with targeted deletion of Cyp8b1 (C57/BL6/Sv129 mixed background) were created as reported previously (7). ApoE KO mice on a pure C57/BL6 background were obtained from Taconic, Hudson, NY. The FXR agonist GW4064 was synthesized by Synthecol, Inc., Forskningsparken Ideon, Lund, Sweden, and was suspended in 1% methylcellulose before administration. [5,6-3H]β-sitostanol was obtained from American Radiolabel Chemicals, Inc., St. Louis, MO and [4-13C] cholesterol from Amersham Biosciences, Sweden.

Two different diets were given: chow containing 0.025% cholesterol (w/w), and water was available ad libitum. Three parallel series of experiments were obtained by heterozygous breeding, and homozygous apoE KO animals were also generated from the same heterozygous breeding. Groups of four to six males aged 10–20 weeks were aged once daily for 6 days with either the agonist (50 mg/kg/day) or the vehicle (1% methylcellulose). Animals were euthanized by CO2 anesthesia, and the liver and or the vehicle (1% methylcellulose). Animals were euthanized by

In the ascending aorta, the hepatic acyl-CoA transferase:cholesterol acyltransferase (ACAT) enzymatic activity in liver tissue was determined in microsomal preparations from pooled samples as previously described (14). Piripirepine A, a highly selective ACAT2 inhibitor (14, 15), was used to differentiate between ACAT1 and ACAT2 activity.

mRNA determination by quantitative real-time PCR

Total RNA was isolated with the Quick Prep Total RNA Extraction Kit (Amersham Biosciences, Sweden). Isolation of enterochromaffin RNA was performed from scraped mucosal cells collected
from the jejunum (the middle third of the small intestine) and ileum (the distal third) and suspended in Trizol (Invitrogen). Oligo-dT-primed cDNA synthesis was carried out on 1 µg of total RNA using the High-Capacity Reverse TranscripTase Kit (Applied Biosystems). Quantification of pooled cDNA was performed with real-time PCR using fluorochrome-labeled TaqMan probes. Forty-five selected genes were analyzed on a 48-well-format low-density TaqMan array, utilizing the 7900 HT sequence detection system (Applied Biosystems). For measurements of individual Cyp7a1 and HMG-CoA reductase levels, Power SYBR® Green PCR Master Mix (Applied Biosystems) was used with primers overlapping exon-exon boundaries. For detection, Applied Biosystems PRISM 7700 Sequence Detection System instrument and software were used. As an internal standard, mouse hypoxanthine phosphoribosyl transferase was selected.

Statistical analysis

Data are presented as means ± SEM. Statistical analysis was performed with STATISTICA software (StatSoft). The significance of differences was tested by two-way ANOVA, followed by posthoc comparison according to the LSD test. A P value <0.05 was considered statistically significant.

RESULTS

Effects of CA depletion on atherosclerosis development

To accelerate the development of atherosclerosis, apoE KO and DKO mice were fed the cholesterol diet for 5 months. Microscopically, typical atherosclerotic lesions occurred in the most proximal part of the ascending aorta, estimated to represent approximately 30% of the aortic area in the apoE KO mice within a zone ranging from 100 to 800 µm distal to the aortic valves. In the DKO animals, the lesion area was approximately 50% smaller compared with the apoE KO mice (Fig. 1). To evaluate any qualitative differences in the atherosclerotic lesions, such as the cellular response, the expressions of various cell surface markers were investigated. No statistically significant difference could be found for the percentage of cells positive for the markers CD3 and I-AK when comparing the plaques in the DKO mice to those in the apoE KO mice (Table 1). The stained area for the CD68 marker was approximately 30% larger in the DKO mice, whereas no differences were seen for CD106.

Effects of CA depletion on serum lipoproteins

When comparing the apoE KO mice to the DKO mice, the former always had higher levels of FC and CE in the plasma (Table 2). This was especially prominent in the mice fed the cholesterol diet for 5 months, inasmuch as the levels of CE observed in the apoE KO mice were more than 2-fold higher (18.2 vs. 8.2 mmol/l) as compared with the DKO mice. That the dietary treatment also increased the levels of CE in the DKO animals could be seen when comparing their values with those of the DKO mice fed chow diet for 2 weeks (8.2 vs. 3.0 mmol/l). More than 90% of the FC and CE occurred in the apoB-containing lipoprotein fractions, which most probably consisted of chylomicrons and chylomicron remnants (Fig. 2). Alterations in the apoA-I-containing fractions (HDL) were modest. No significant differences were detected for the triglyceride levels in the lipoprotein profiles when referring to diet regimens and feeding periods.

Effects of CA depletion on the intestinal cholesterol absorption

DKO mice fed the chow diet absorbed approximately 60% less cholesterol compared with the apoE KO animals (see supplementary Fig. 1), which is a pattern similar to that found in Cyp8b1 KO mice when compared with their corresponding WT animals (7, 11). In animals fed the cholesterol diet for 2 weeks, the total percentage of absorbed cholesterol was reduced in both strains of mice, but still the DKO animals showed approximately 50% less cholesterol absorption (see supplementary Fig. 1).

Effects of CA depletion on the composition of bile and hepatic cholesterol

ApoE KO mice fed the cholesterol diet for either 2 weeks or 5 months showed a prominent increase in their hepatic CE content, in contrast to the DKO mice, which displayed essentially the same levels as found in apoE KO and DKO animals fed a chow diet (Fig. 3A). Thus, the phenotype observed in the Cyp8b1 KO mice, decreased intestinal cholesterol absorption and lower hepatic CE content, was preserved in the DKO mice. Determination of the hepatic activity of the cholesterol-esterifying enzyme ACAT2 showed that it was much higher in the apoE KO mice than in the DKO mice after 5 months of cholesterol diet feeding, but in animals fed the cholesterol diet for 2 weeks, the difference was not significant (Fig. 3B). No changes were recorded for ACAT1 activity (data not shown). As expected, the percentage of CA in primary bile was negligible in the DKO mice, which consistently was compensated for by an increase of β-muricholic acid and chenodeoxycholic/α-muricholic acid (Table 3).

Effects of CA depletion on bile acid and cholesterol metabolism

The hepatic CA/total cholesterol ratio, an indicator of bile acid synthesis, was significantly higher in the DKO compared with the apoE KO mice in all diet groups (Fig. 4A). A similar pattern was found for Cyp7a1 mRNA expression (Fig. 4B), indicating that the bile acid synthesis was much more intensive in the DKO animals. Furthermore, the cholesterol diet did not change this pattern. No differences were recorded for the mRNA levels of Cyp27 and Cyp7b1 in DKO or apoE KO mice when comparing the chow diet to the cholesterol diet in the 2 week feeding period (see supplementary Fig. II). Determination of the hepatic lathosterol/total cholesterol ratio, reflecting de novo cholesterol synthesis in the liver, showed that there were no significant differences between DKO and apoE KO mice when fed a chow diet (Fig. 4C). In apoE KO mice fed the cholesterol diet, the ratio of lathosterol/total cholesterol was reduced compared with the apoE KO mice fed the chow diet. In contrast, the DKO mice showed no significant differences after 2 weeks of cholesterol diet feeding, whereas a modest reduction was obtained after 5 months. The mRNA levels for HMG-CoA reductase demonstrated a
compared with apoE KO mice after 2 weeks of cholesterol diet, possibly due to less accumulation of cholesterol in the liver. A similar pattern was observed after 5 months of cholesterol diet (data not shown).

Of the genes occurring in the distal ileum, $Fgf15$ mRNA levels were reduced more than 50% in the DKO mice compared with the apoE KO mice after both 2 weeks and 5 months of the cholesterol diet. This would be a consequence of abolished CA synthesis in the DKO mice, inasmuch as $Fgf15$ gene expression is upregulated via FXR, and furthermore, it would explain why $Cyp7a1$ mRNA levels were higher in the DKO mice. No differences were observed in the mRNA expressions for $Abcg5/g8$, $Abca1$, $Lxr$ and $H9251$, or $Fxr$ in jejunum or for $Ibabp$ or $Asbt$ in ileum with respect to genotype after 2 weeks of cholesterol diet feeding (see supplementary Figs. II, III). A reduction was observed in hepatic $Abcg5/g8$ mRNA expression in DKO mice after 2 weeks of cholesterol diet, possibly due to less accumulation of cholesterol in the liver. A similar pattern was observed after 5 months of cholesterol diet (data not shown).

Effects of FXR agonist treatment on CA-depleted mice

To differentiate between the micellar and FXR signaling effects of CA, groups of DKO mice were treated either with dietary CA (0.2% w/w) or with the synthetic FXR agonist GW4064 (50 mg/kg/day). Administration of GW4064 did not change the intestinal cholesterol uptake, whereas
addition of CA markedly increased the intestinal cholesterol absorption to the same level seen in apoE KO animals (Fig. 5A). Treatments with both GW4064 and CA upregulated the Fgf15 mRNA in the ileum, but the strongest response was obtained with the synthetic agonist (Fig. 5B). In addition, a prominent induction of Shp mRNA was found for GW4064, but much less for CA. Expression of Npc1l1 in jejenum and ileum was essentially unchanged by the CA and GW4064 treatment in the DKO mice, and mRNA levels for Abcg5/g8 were also unaffected by the GW4064 treatment. Thus, the cholesterol absorption in this experiment did not correlate with the Npc1l1 expression, but rather with the presence or absence of CA, which might reflect that the main effect of CA for cholesterol absorption is due to its micellar-forming properties.

GW4064 did not induce any changes in the total plasma cholesterol concentrations compared with the vehicle-gavaged animals, but, in contrast, CA restored the total cholesterol levels in all plasma fractions to a pattern similar to that occurring in untreated apoE KO mice (data not shown). Nor did the FXR agonist treatment affect the levels of hepatic CE when compared with vehicle-treated animals (data not shown). Treatment with both CA and GW4064 decreased the mRNA levels for HMG-CoA reductase, HMG-CoA synthase, and Cyp7a1, indicating a down-regulation of cholesterol and bile acid synthesis (Fig. 5C). GW4064 administration also reduced the amount of chenodeoxycholic/α-muricholic acid in the bile, whereas the amount of β-muricholate increased (Table 3).

**DISCUSSION**

Not only do bile acids facilitate the uptake of dietary lipids, they also exert endocrine and paracrine effects within various areas of the metabolism. In addition to the micellar-forming ability in the intestine, CA also functions as a specific ligand for FXR (2). Mice with an inhibited synthesis of CA have several phenotypic features: decreased cholesterol absorption, increased synthesis of bile acids and cholesterol, and resistance to hepatic accumulation of CEs. The present study is the first to demonstrate the effects of abolished CA synthesis on lipid metabolism and atherosclerotic progression in the apoE-deficient mouse, a well-known animal model for the studies of cardiovascular diseases.

Compared with apoE KO animals, the extent of atherosclerotic lesions in DKO animals was 50% less. Analysis of the cellular markers CD106 (VCAM-1), CD3, and I-Aβ in the lesions indicated that no essential differences occurred between the two groups in the local inflammatory process. The DKO mice displayed lower levels of cholesterol in their apoB-containing lipoproteins, upregulated bile acid synthesis, and less accumulation of CE in the liver. Because no significant changes occurred in the cholesterol levels of the apoA-I-containing lipoprotein particles, we can assume that the major part of the absorbed cholesterol ends up in apoB-containing lipoproteins. This particular fraction consists mainly of chylomicron remnants, inasmuch as the deficiency of apoE seriously hampers their receptor-mediated uptake into the liver, and furthermore, it is reported that the hepatic release of VLDL particles in those mice is decreased (16). Compared with the apoE KO animals, the DKO mice displayed approximately 50% lower levels of plasma CE in their apoB-containing fractions, which seems to be due to the diminished uptake of intestinal cholesterol in these mice. Actually, DKO mice fed the cholesterol diet displayed levels of CE in their apoB-containing lipoproteins similar to those seen in apoE KO mice on the chow diet (containing 0.25% cholesterol or 1/8 of the cholesterol amount in the cholesterol diet). As a consequence, we could estimate that depletion of CA confers resistance to a diet containing at least eight times more cholesterol.

The relationship between intestinal cholesterol absorption and atherosclerotic development in apoE KO mice has been investigated using several approaches: treatment with ezetimibe (17) or genetic depletion of NPC1L1 (18) or ACAT2 (19). In all cases, a prominent reduction of
nevertheless remarkable that the intestinal cholesterol absorption in our DKO mice was not reduced even more; Wang et al. (24) demonstrated that mice with high levels of muricholic acids in their bile (80–85%) only absorbed 11–12% of luminal cholesterol. One possible explanation could be that the bile acid pool of the Cyp8b1-deficient animals is expanded and contains slightly more hydrophobic bile acid and less (75%) muricholic acid. An increased uptake of bile acid in the distal ileum is less probable, inasmuch as no alterations were observed in the mRNA for bile acid transporters.

Earlier studies have suggested that FXR agonist treatment could be responsible for suppression of intestinal cholesterol absorption in the mouse (25). However, treatment of DKO mice with the synthetic FXR agonist GW4064 cholesterol absorption was demonstrated, accompanied by a significant decrease of atherosclerotic lesions. Ezetimibe treatment and genetic depletion of NPC1L1 probably affect the same uptake mechanism within the enterocyte, and the mode of action is limited to the direct absorptive process. Targeted disruption of Npc1l1 in apoE KO mice reduces the total cholesterol absorption by 60–70% (20, 21) and the atherosclerotic lesions by >90% (22). Elimination of CA decreases the uptake to barely half, corresponding to a total cholesterol absorption of around 30%, and reduces atherosclerotic lesions by 50%. However, the atheroprotective effect seems to vary in different apoE mouse models, inasmuch as even a modest reduction of 41% of the intestinal cholesterol absorption achieved a 70% decrease of the atherosclerosis formation (23). It is

Fig. 2. Lipoprotein profiles in serum by size-exclusion chromatography. Relative cholesteryl ester content in serum of apoE KO and DKO animals fed a chow diet for 2 weeks or cholesterol diet for 2 weeks or 5 months.
did not restore the intestinal absorptive capacity (or induce any changes in the intestinal cholesterol absorption), whereas in fact, addition of 0.2% CA did. Our results showed that both GW4064 and CA increased the transcription of several target genes for FXR, such as Shp and Fgf15, whereby the peptide coded for by the latter would down-regulate the Cyp7a1 transcription (11). The major function for CA in the cholesterol absorptive process would thus be restricted to the formation of appropriate micelles, rather than ability to modulate the expression of FXR-regulated genes. This view is supported by the finding that the intestinal expression of Npc1l1 was unaffected by the administration of CA or GW4064. Furthermore, Abcg5/g8 expression was not affected by GW4064 in the DKO mice, suggesting that the FXR agonist did not affect the net uptake of cholesterol into the enterocytes.

Our results show that four characteristic features of the Cyp8b1 KO phenotype were preserved in the DKO animals: upregulated bile acid and de novo cholesterol synthesis in the liver, reduced intestinal cholesterol absorption, and reduced accumulation of hepatic CE. Even if the DKO mice synthesize larger quantities of bile acids from cholesterol, most of the production consists of muricholic acid, being much less efficient in carrying out micellar formation, and thus cholesterol absorption (24, 26), and FXR-mediated signaling. This might explain why DKO mice have lower hepatic CE levels and decreased ACAT2 activity, compared with apoE KO mice, even when the former are kept on a cholesterol diet and have a higher rate of cholesterol synthesis. Elimination of the FXR agonist CA seems to decrease the production of FGF15, which might be the main reason why Cyp7a1 is upregulated, a finding that our results showed.

**TABLE 3.** Bile acid composition of gallbladder bile

<table>
<thead>
<tr>
<th>Bile Acid Composition (% of Total Bile Acids)</th>
<th>DCA</th>
<th>CDCA + α-Muricholic Acid</th>
<th>CA</th>
<th>UDCA</th>
<th>β-Muricholic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow Diet 2 weeks</td>
<td>ApoE KO &lt;1</td>
<td>5.3 ± 1.7</td>
<td>52.0 ± 3.8</td>
<td>&lt;1</td>
<td>42.5 ± 4.0</td>
</tr>
<tr>
<td>Cholesterol diet 2 weeks</td>
<td>DKO</td>
<td>24.8 ± 1.9*</td>
<td>&lt;1*</td>
<td>3.5 ± 2.0*</td>
<td>71.7 ± 3.5*</td>
</tr>
<tr>
<td>Chow Diet 6 days</td>
<td>DKO</td>
<td>3.6 ± 1.8</td>
<td>3.4 ± 1.4</td>
<td>72.9 ± 9.4</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>Chow Diet 6 days + 0.2% CA</td>
<td>DKO</td>
<td>3.6 ± 1.8</td>
<td>3.4 ± 1.4</td>
<td>72.9 ± 9.4</td>
<td>1.7 ± 1.3</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM (n = 3–5). Values represent percent (%) of total gallbladder bile acids. Before euthanization, the mice were fasted for 4 h. CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; DCA, deoxycholic acid. *Statistically significant difference from apoE KO with the same diet. **Statistically significant difference from apoE KO chow diet for 6 days, P < 0.05.
which is in accordance with the obtained hepatic C4/total cholesterol values. Our results also demonstrated that the bile acid synthesis of the apoE KO mice was not enhanced by the cholesterol diet, which might explain their hepatic accumulation of CE.

Addition of cholesterol to the diet is reported to increase Cyp7a1 mRNA expression and consequently, bile acid synthesis (27). However, our animals fed the 0.2% cholesterol diet did not upregulate their Cyp7a1 expression. One reason may be that previous studies utilized diets containing 2% cholesterol, whereas diets with 0.2% cholesterol have not been extensively applied. With treatment of WT and apoE KO mice with a 2% cholesterol diet, the mean values of the Cyp7a1 mRNA levels doubled, although statistical significance was not reached, owing to large interindividual variations, whereas the 0.2% cholesterol diet did not induce any apparent changes (see supplementary Fig. V). The Cyp7a1 mRNA levels of the DKO mice were not affected by the diets, probably because Cyp7a1 already was highly upregulated in these mice. Even if the 0.2% cholesterol diet did not increase the mRNA levels of Cyp7a1, the dose was nevertheless able to induce plaque formation in the apoE KO and DKO animals.

The effect of ezetimibe in humans is not as prominent as in mice; treatment of mildly hypercholesterolemic patients reduced the cholesterol absorption an average of 54% (28). Nevertheless, today, inhibition of cholesterol absorption by ezetimibe is one of the major therapeutic alternatives for treatment of cardiovascular diseases, although until now, long-term studies in large patient groups have not been available. In recent clinical studies, the impact on cardiovascular events with ezetimibe treatment was less than expected (29). In humans, large interindividual variations exist between the proportions of CA and chenodeoxycholic acid, the other major bile acid (30), and studies evaluating the effect of CA on intestinal cholesterol absorption show contradictory results. In some investigations, administration of CA did not change the absorption (31, 32), whereas in others, an increase was reported (33). Reduced CA production by administration of an appropriate inhibitor to the CYP8B1 P-450 cytochrome may offer yet another possibility to diminish cholesterol.

Fig. 4. Effects of CA depletion on hepatic bile acid and cholesterol synthesis. A: 7α-Hydroxy-4-cholesten-3-one (C4) content, related to hepatic total cholesterol levels. * Statistically significant difference from apoE KO mice treated with the corresponding diet, \( P < 0.05 \). B: mRNA levels of the hepatic genes Cyp7a1 and HMG-CoA reductase, related to the corresponding mRNA levels of apoE KO mice fed a chow diet for 2 weeks (2 W). * Statistically significant difference from apoE KO mice fed a chow diet for 2 weeks, \( P < 0.05 \). † Statistically significant difference from DKO mice fed a chow diet for 2 weeks, \( P < 0.05 \). C: Hepatic lathosterol concentrations, as related to hepatic total cholesterol content. * Statistically significant difference from apoE KO mice treated with the corresponding diet, \( P < 0.05 \). Data are presented as mean ± SEM.
chlorate depletion reduces atherosclerosis.

REFERENCES


Fig. 5. Effects of treatment of DKO and apoE KO animals with the farnesoid X receptor agonist GW4064 or 0.2% CA. The animals were treated for 6 days with GW4064 (50 mg/kg/day), vehicle (1% methylcellulose), or CA (0.2%). A: Intestinal cholesterol absorption. * Statistically significant difference from apoE KO, P < 0.05. B: Relative mRNA levels of intestinal genes involved in cholesterol transport and signaling in pooled samples from jejunum (je) and ileum (il) of the small intestine. C: Relative mRNA levels of genes involved in cholesterol and bile acid synthesis and transport in pooled hepatic samples. Data are presented as mean ± SEM.

absorption. Presently, it is not possible to foresee all the effects in humans following a decreased synthesis of CA, although reduced cholesterol absorption would be expected. Because chenodeoxycholic acid seems to be the primary FXR ligand in humans (34), it is difficult to speculate about the metabolic consequences of such an approach, or about whether higher proportions of chenodeoxycholic acid in the bile might alter the release of FGF19 with consequences for bile acid production. Inhibition of CYP8B1 might also be considered for treatment of other diseases with disturbed cholesterol homeostasis like gallstone disease, because gallstone formation might be prevented by depleting CA (11).

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