H1-antihistamines exacerbate high-fat diet-induced hepatic steatosis in wild-type but not in apolipoprotein E knockout mice

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Submitted 17 January 2014; accepted in final form 11 May 2014

Raveendran VV, Kassel KM, Smith DD, Luyendyk JP, Williams KJ, Cherian R, Reed GA, Flynn CA, Csakany IL, Lickteig AL, Pratt-Hyatt MJ, Klaassen CD, Dileepan KN. H1-antihistamines exacerbate high-fat diet-induced hepatic steatosis in wild-type but not in apolipoprotein E knockout mice. Am J Physiol Gastrointest Liver Physiol 307: G219–G228, 2014. First published May 22, 2014; doi:10.1152/ajpgi.00027.2014. —We examined the effects of two over-the-counter H1-antihistamines on the progression of fatty liver disease in male C57Bl/6 wild-type and apolipoprotein E (ApoE)−/− mice. Mice were fed a high-fat diet (HFD) for 3 mo, together with administration of either cetirizine (4 mg/kg body wt) or fexofenadine (40 mg/kg body wt) in drinking water. Antihistamine treatments increased body weight gain, gonadal fat deposition, liver weight, and hepatic steatosis in wild-type mice but not in ApoE−/− mice. Lobular inflammation, acute inflammation, and necrosis were not affected by H1-antihistamines in either genotype. Serum biomarkers of liver injury tended to increase in antihistamine-treated wild-type mice. Serum level of glucose was increased by fexofenadine, whereas lipase was increased by cetirizine. H1-antihistamines reduced the mRNA expression of ApoE and carbohydrate response element-binding protein in wild-type mice, without altering the mRNA expression of sterol regulatory element-binding protein 1c, fatty acid synthase, or ApoB100, in either genotype. Fexofenadine increased both triglycerides and cholesterol ester, whereas cetirizine increased only cholesterol ester in liver, with a concomitant decrease in serum triglycerides by both antihistamines in wild-type mice. Antihistamines increased hepatic levels of conjugated bile acids in wild-type mice, with the effect being significant in fexofenadine-treated animals. The increase was associated with changes in the expression of organic anion transport polypeptide 1b2 and bile salt export pump. These results suggest that H1-antihistamines increase the progression of fatty liver disease in wild-type mice, and there seems to be an association between the severity of disease, presence of ApoE, and increase in hepatic bile acid levels.

HISTAMINE IS A BIOGENIC MONOAMINE that exerts many immunological and physiological functions, including hematopoiesis, vasodilation, allergic response, neurotransmission, and gastric acid release (3, 13, 14). The physiological effects of histamine are mediated through a family of four G protein-coupled receptors, namely histamine H1 receptor (H1R), H2R, H3R, and H4R (28). In addition to its well-recognized role in the regulation of many vascular and immunological functions, histamine regulates the homeostasis of energy intake and expenditure (32). The histaminergic neurons are concentrated in the tuberomamillary nucleus of the posterior hypothalamus and control appetite through the H1R (27, 32, 39). Furthermore, histamine is known to regulate lipid and carbohydrate metabolism (10, 24, 36). Recent reports suggest that deletion of H1R or H2R genes increases nonalcoholic fatty liver disease (NAFLD) in mice fed a high-fat diet (HFD) (38). Fatty liver disease is characterized by a wide spectrum of liver abnormalities ranging from simple steatosis, to steatohepatitis, cirrhosis, and hepatocellular carcinoma (2). The prevalence of NAFLD is dependent on ethnicity, sex, existence of hypertension, diabetes, and higher body mass index (40). Because histidine decarboxylase knockout and H1R knockout mouse models exhibit glucose intolerance and abdominal adiposity, it is suggested that histamine signaling though histamine receptors can regulate obesity (10, 24). H1-antihistamines are used by millions of people for controlling histamine-mediated allergic symptoms, but their chronic use is reported to increase weight gain in humans (30).

Apolipoprotein E (ApoE) is primarily produced by the liver, and its major function is to facilitate the transport of triglycerides and cholesterol from chylomicron remnant, very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) to liver and peripheral tissues (15). Low-density lipoprotein receptors (LDLr) in the liver take up triglycerides and cholesterol from ApoE-bound lipoproteins. ApoE−/− mice are unable to efficiently clear lipids from the circulation, which leads to the development of hypertriglyceridemia, hypercholesterolemia, and spontaneous atherosclerosis (25, 29). Although both H1R and ApoE have been implicated in lipid homeostasis, their interrelations in the pathogenesis of fatty liver disease have not been studied.

Bile acids play a significant role in dietary lipid absorption, cholesterol homeostasis, and drug disposition (6, 12). Therefore, alterations in bile acid homeostasis may be an important factor in the pathophysiology of diseases associated with altered lipid metabolism, obesity, and hepatic steatosis. Clinical studies have shown that HFD and obesity affect biliary excretion of drugs (12). Furthermore, in rodents, HFD-induced obesity has been found to be associated with changes in the expression of genes involved in the biliary excretion of drugs (5, 21). The objectives of this study were to examine the effects of cetirizine and fexofenadine, two commonly used therapeutic H1-antihistamines, on HFD-induced hepatic steatosis and to
therapeutic doses for human adults adjusted for the mouse (31). The water intake was measured every third day, and food intake was measured twice during the course of the study. Mice were weighed at the beginning and the end of the study. All animal experiments complied with the guidelines published by the National Institute of Health and were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center.

### MATERIALS AND METHODS

#### H1-antihistamines and reagents.

Cetirizine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO). Allegra (fexofenadine HCl tablet, 180 mg/antihistamine, over the counter) was used as the source of fexofenadine. Fexofenadine tablets were ground, dissolved in 5 ml water/day. The manufacturer’s protocol. One microgram of total RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (ab136853; Abcam, Cambridge, MA) was used. After being snap-frozen in liquid nitrogen, livers were stored at −80°C until assayed. In brief, 50–100 mg of the liver tissue was homogenized in 1 ml 5% Triton-X 100 in PBS and then slowly heated to 80°C in water bath for 5 min. The samples were cooled down and again heated to solubilize triglycerides into solution. The samples were centrifuged for 5 min, and supernatants were diluted 10-fold with distilled water and analyzed colorimetrically following the manufacturer’s protocol.

The total cholesterol and free cholesterol content of the liver was measured colorimetrically using a Cholesterol assay kit (STA-384; Cell Biolabs, San Diego, CA). Cholesterol ester was calculated by subtracting free cholesterol from total cholesterol levels. Ten milligrams of liver tissue was extracted with 200 μl of chloroform:methanol (2:1) in a glass homogenizer. The extract was centrifuged at 15,000 × g for 10 min, and the liquid phase was transferred to a new glass tube and air-dried at 50°C under a hood. The dried samples were dissolved in 200 μl of 1 × assay diluent following the protocol provided by the manufacturer. Analyses of mRNA expression in liver. Total RNA was extracted from livers using TRIzol Reagent (Molecular Research Center, Cincinnati, OH) or RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. One microgram of total RNA was reverse transcribed with the High-Capacity cDNA Reverse Transcription Kit (ab136853; Abcam, Cambridge, MA) was used. After being snap-frozen in liquid nitrogen, livers were stored at −80°C until assayed. In brief, 50–100 mg of the liver tissue was homogenized in 1 ml 5% Triton-X 100 in PBS and then slowly heated to 80°C in water bath for 5 min. The samples were cooled down and again heated to solubilize triglycerides into solution. The samples were centrifuged for 5 min, and supernatants were diluted 10-fold with distilled water and analyzed colorimetrically following the manufacturer’s protocol.

### Histopathological analysis of liver tissue.

Paraffin-embedded left lateral lobes of livers of mice were sectioned at 5-μm thickness and stained with hematoxylin and eosin. The severity of liver steatosis and other lesions were scored by two pathologists (R.C. and K.J.W) independently in a blinded manner (34). The degree of macrosteatosis and microsteatosis were semi-quantitatively scored on a scale of 0 to 3 (where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe). Acute inflammation and lobular inflammation were scored on a scale of 0 to 3 (where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe), and spotty necrosis was graded on a scale of 0 to 4 (where 0 = none, 1 = occasional single necrotic cell, 2 = <10 foci of single cell necrosis per high-powered field (HPF); 3 = ≥10 foci of single cell necrosis per HPF; and 4 = focally confluent necrosis).

#### Biochemical measurement of triglycerides and cholesterol in liver.

For hepatic triglyceride measurements, a Triglyceride Quantification Kit (ab65336; Abcam, Cambridge, MA) was used. After being snap-frozen in liquid nitrogen, livers were stored at −80°C until assayed. In brief, 50–100 mg of the liver tissue was homogenized in 1 ml 5% Triton-X 100 in PBS and then slowly heated to 80°C in water bath for 5 min. The samples were cooled down and again heated to solubilize triglycerides into solution. The samples were centrifuged for 5 min, and supernatants were diluted 10-fold with distilled water and analyzed colorimetrically following the manufacturer’s protocol.

#### Concentrations of cetirizine and fexofenadine in serum (ng/ml)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cetirizine</th>
<th>Fexofenadine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>BD</td>
<td>BD</td>
</tr>
<tr>
<td>CET</td>
<td>425.7 ± 40.3</td>
<td>709.9 ± 145.9</td>
</tr>
<tr>
<td>FEX</td>
<td>11.5 ± 4.3</td>
<td>8.4 ± 0.3</td>
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<table>
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<tr>
<th>ApoE–/–</th>
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<tbody>
<tr>
<td>CTL</td>
<td>BD</td>
<td>BD</td>
</tr>
<tr>
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<tr>
<td>FEX</td>
<td>11.5 ± 4.3</td>
<td>8.4 ± 0.3</td>
</tr>
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</table>

Applicable values are means ± SE. BD, below detection limit; CTL, control; CET, cetirizine; FEX, fexofenadine.
transcribed into single-strand cDNA using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) following the manufacturer’s procedure. The expression of lipogenic genes, including sterol regulatory element-binding protein 1c (SREBP1c), carbohydrate regulatory element-binding protein (ChREBP), and fatty acid synthase (FAS); the lipid transport-related genes, including ApoB100, ApoE, and LDLr; and bile acid transport genes, including organic anion transport polypeptide 1B2 (Oatp1b2), bile salt export pump (Bsep), and Na$^+$-taurocholate cotransporting polypeptide (Ntcp), were quantified by real-time qRT-PCR using SYBR Green PCR Master Mix and gene-specific primers. The primers were designed using Primer Express Software v3.0 from Applied Biosystems or Primer3Web, v4.0.0 from MIT (Cambridge, MA). The primer sequences are given in Table 1.

Quantification of bile acids in liver. One hundred milligrams of liver samples were used for the extraction of bile acids. The samples were spiked with internal standards [40 μg/ml d4-CDCA in methanol] and bile acids were extracted and quantified by ultraperformance liquid chromatography-tandem mass spectrometry as previously described (1, 42).

Quantification of cetirizine and fexofenadine in serum. Serum samples collected from three untreated control mice and six mice treated with either cetirizine or fexofenadine were processed by protein precipitation using acetonitrile (Table 2). The purified samples were subjected to reverse-phase liquid chromatography-tandem mass spectrometry for quantification of cetirizine or fexofenadine (9).

Serum chemistry and lipid profiles. Serum chemistry and lipid profile were analyzed at the Veterinary Laboratory Resources (Overland Park, KS).

Statistical analyses. One-way or two-way ANOVA followed by Dunnett’s multiple-comparison test employing GraphPad Prism version 5.04 (San Diego, CA) was used for statistical analyses as applicable. Results are represented as means ± SE, and $P \leq 0.05$ is considered significant.

RESULTS

H1-antihistamines increase body weight gain, liver weight, and gonadal fat deposition in HFD-fed wild-type mice but not in ApoE$^{−/−}$ mice. The body weight gains in wild-type mice maintained on a HFD together with either cetirizine or fexofenadine for 3 mo were significantly higher than the control group receiving only HFD (Fig. 1A). In contrast, compared with wild-type controls, the weight gain was significantly lower in all groups of ApoE$^{−/−}$ mice irrespective of their treatments. The food and water intake were comparable in all groups of mice (Table 3). The significant increase in liver and gonadal adipose tissue weights accounted for 32 and 38% of the body weight gain in cetirizine- and fexofenadine-treated wild-type mice, respectively (Fig. 1, B and C).

H1-antihistamines increase macrovesicular steatosis in wild-type mice but not in ApoE$^{−/−}$ mice. The wild-type mice treated with H1-antihistamines had significantly increased macrovesicular steatosis compared with untreated control mice (Fig. 2, A and B). H1-antihistamines did not alter microsteatosis, lobular

### Table 3. Daily intake of water and food

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wild-type</th>
<th>ApoE$^{−/−}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water, ml</td>
<td>Food, g</td>
</tr>
<tr>
<td>CTL</td>
<td>7.5 ± 0.8</td>
<td>2.9 ± 0.0</td>
</tr>
<tr>
<td>CET</td>
<td>7.4 ± 0.7</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>FEX</td>
<td>7.4 ± 0.7</td>
<td>2.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.
inflammation, acute inflammation, or spotty necrosis in wild-type mice or in ApoE−/− mice (Fig. 2B). The lobular inflammation and acute inflammation as well as necrosis were inherently higher in ApoE−/− mice compared with wild-type mice. The number of animals with increased steatosis was higher in antihistamine-treated mice (Fig. 2C). Summary of frequency of number of animals with different degree of steatosis evaluated by two different pathologists is given in Table 4.

Fexofenadine increases triglyceride levels, and cetirizine increases only cholesterol ester in liver of wild-type mice. To examine whether histopathological evidence of steatosis was accompanied by a biochemical increase in hepatic lipid levels, we measured triglycerides, total cholesterol, and free cholesterol and calculated cholesterol ester in the liver tissue. The hepatic triglyceride and cholesterol ester levels were significantly increased in fexofenadine-treated wild-type mice (Fig. 3A and D), without altering total cholesterol (Fig. 3B) and free cholesterol levels (Fig. 3C). On the other hand, cetirizine significantly increased the level of hepatic cholesterol ester only (Fig. 3D).

Effect of H1-antihistamines on serum lipid and chemistry profile. Figure 4 illustrates the effect of H1-antihistamines on serum lipid profiles of wild-type and ApoE−/− mice. H1-antihistamine treatments did not alter serum levels of HDL, LDL, or total cholesterol in either wild-type or ApoE−/− mice. In ApoE−/− mice, LDL, triglycerides, and total cholesterol levels in the serum were markedly higher, and HDL levels were significantly lower compared with wild-type mice. It is noteworthy that serum levels of triglycerides, but not HDL, LDL, or total cholesterol, was significantly reduced by H1-antihistamine treatments in wild-type mice (Fig. 4B).

Figure 5, A–C, demonstrates the serum levels of alanine aminotransferase, aspartate aminotransferase (markers of hepatocellular necrosis), and alkaline phosphatase (biomarker of
biliary tract injury), respectively. All these liver enzyme biomarkers showed a trend toward an increase in sera of wild-type mice treated with H1-antihistamines. Serum glucose levels were significantly higher in fexofenadine-treated, but not in cetirizine-treated, wild-type mice (Fig. 5D). Interestingly, lipase levels were significantly higher in cetirizine-treated mice (Fig. 5E).

Changes in expression of lipogenic and lipid transport genes by H1-antihistamines. Livers from all groups of mice were analyzed for the expression of genes associated with lipogenesis and lipid transport. The expression of mRNAs encoding SREBP1c, FAS, ApoB100, and LDLr were not altered by H1-antihistamine treatment in wild-type mice (Fig. 6, A, C, D, and F). Interestingly, hepatic ChREBP and ApoE mRNA levels were significantly reduced by antihistamine treatment in wild-type mice (Fig. 6, B and E).

Antihistamine treatments did not alter the expression of mRNA for SREBP1c, ChREBP, FAS, ApoB100, or LDLr in ApoE−/− mice (Fig. 6, G–K).

H1-antihistamines alter bile acid homeostasis in liver. Figure 7 shows the amount of conjugated and unconjugated bile acids in livers of wild-type mice treated with H1-antihistamines. Compared with control, fexofenadine treatment significantly increased conjugated (Fig. 7A), but not unconjugated, bile acids (Fig. 7B). Although not statistically significant, cetirizine treatment tended to increase conjugated bile acid levels. Fexofenadine, but not cetirizine, significantly increased taurine conjugates of 7α-muricholic acid (7αMCA), 7β-muricholic acid (7βMCA), cholic acid, and ursodeoxycholic acid. Both antihistamines significantly increased taurochenodeoxycholic acid. Other conjugated and unconjugated

Table 4. Frequency of animals with steatosis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Score</th>
<th>Pathologist 1</th>
<th>Pathologist 2</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CL</td>
<td>MZ</td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>8/8</td>
<td>1/8</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>—</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>Normal</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>2/8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>6/8</td>
<td>3/8</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>Normal</td>
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<tr>
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<td>Mild</td>
<td>1/8</td>
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<tr>
<td></td>
<td>Moderate</td>
<td>7/8</td>
<td>4/8</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>—</td>
<td>4/8</td>
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Hematoxylin and eosin-stained histological sections were independently scored by two pathologists in a blinded fashion. Each slide was examined at three regions of liver lobule for degree of steatosis. The frequency of animals falling in each category ranging from normal to severe is tabulated. Total number of animals evaluated is 8. CL, centrilobular; MZ, mid zone; PP, periportal.

Fig. 3. Effect of H1-antihistamines on hepatic lipid profile of wild-type mice. Hepatic triglycerides (A), total cholesterol (B), free cholesterol (C), and cholesterol ester levels (D) were calculated on basis of per gram liver tissue. Each value presented is the mean ± SE. *P < 0.05, **P < 0.01, N = 7 or 8.
bile acids remained unaltered by H1-antihistamine treatments (data not shown).

H1-antihistamines alter hepatic bile acid transport gene expression. Transporters play a pivotal role in the enterohepatic circulation of bile acids. In liver, conjugated bile acids are taken up from the portal blood by the basolateral transporter Ntcp (7, 18) and unconjugated bile acids by Oatp1b2 (7). Bile acids and their conjugates are excreted into bile by Bsep (17, 35). The effects of H1-antihistamines on hepatic mRNA expression of these bile acid transporters in wild-type mice are presented in Fig. 8. Compared with control, fexofenadine treatment increased the mRNA expression of Oatp1b2 (Fig. 8A), whereas both cetirizine and fexofenadine decreased the mRNA expression of Bsep (Fig. 8B). The mRNA expression of Ntcp was not significantly affected by either antihistamine (Fig. 8C).

DISCUSSION

Cetirizine and fexofenadine are the commonly used H1-antihistamines. It is reported that H1R gene-knockout mice fed a HFD exhibit increased hepatic steatosis, hyperglycemia, and insulin resistance (38). This suggests that histamine signaling via H1R is involved in both lipid and carbohydrate metabolism (38). Here, we show that chronic administration of cetirizine or fexofenadine worsens progression of hepatosteatosis in wild-type mice, but not in ApoE−/- mice. Although both H1-antihistamines increased the fatty liver disease, the effect of fexofenadine was more apparent and was associated with significantly increased levels of glucose and hepatic bile acids.

The role of histamine and H1R in regulating obesity is evident from the fact that histidine decarboxylase knockout or H1R knockout mice are obese when fed a HFD compared with...
Fig. 6. Effect of H1-antihistamines on gene expression in liver. Total RNA were isolated from livers and reverse transcribed as detailed in MATERIALS AND METHODS. Specific genes were analyzed, and relative expression is measured by real-time qRT-PCR and represented as mean fold changes ± SE over control.

Livers were analyzed for lipogenic genes in liver of wild-type mice such as shown (A–C). A: sterol regulatory element-binding protein 1c (SREBP1c). B: carbohydrate regulatory element-binding protein (ChREBP). C: fatty acid synthase (FAS). Lipid transport genes in liver of wild-type mice are shown (D–F) D: apolipoprotein B 100 (ApoB100). E: low-density lipoprotein receptor (LDLr). F: ApoE. Expression in liver of ApoE<sup>−/−</sup> mice is shown (G–K) G: SREBP1c. H: ChREBP. I: FAS. J: ApoB100. K: LDLr. *P < 0.05, N = 8 for wild-type and 4 to 6 for ApoE<sup>−/−</sup> mice.
control (10, 24). This finding is further supported by the report showing reduced fat accumulation and adiposity in mice infused with histamine (23). Obesity is closely associated with the development of steatosis and other aspects of the metabolic syndrome (26). In contrast to our expectation (19, 22), H1-antihistamines did not alter food or water intake in wild-type or ApoE−/− mice. A plausible explanation for this observation is the inability of cetirizine and fexofenadine to cross the blood-brain barrier to block H1R signaling in the hypothalamic region that normally suppresses appetite and food intake. Despite the comparable amounts of food and water intake, the H1-antihistamine-treated wild-type mice gained more body weight and increased deposition of gonadal fat than untreated control mice. This observation is in accordance with the findings that chronic use of H1-antihistamines exacerbates obesity in humans (30). ApoE−/− mice have increased circulating levels of histamine compared with wild-type mice (37). The combined effect of increased levels of histamine and lack of ApoE might be responsible for diminished weight gain and gonadal fat deposition in ApoE−/− mice despite similar intake of food and water compared with wild-type mice. The increased body weight and gonadal fat adiposity in wild-type mice on these H1-antihistamine regimens indicate that the rate of energy storage is greater in these animals compared with untreated control mice.

Chronic administration of H1-antihistamines worsened macrosteatosis only in wild-type mice. H1-antihistamines did not significantly alter inflammation and necrosis in either wild-type or ApoE−/− mice. The ApoE−/− mice inherently have increased hepatic inflammation and necrosis compared with wild-type mice (Fig. 2B). It has been established that a severe inflammatory cytokine and chemokine profile, as well as increased expression of adhesion molecules, are present in livers of ApoE−/− mice compared with C57Bl/6 wild-type mice (41). The fact that H1-antihistamine-induced increase in macrosteatosis was seen only in wild-type mice suggests the involvement of ApoE in antihistamine-augmented hepatic steatosis. This contention is supported by the fact that presence of ApoE predisposes mice to fat accumulation and HFD-induced obesity (11, 15). Furthermore, wild-type mice or LDLr-deficient mice carrying functional ApoE exhibit more steatosis than ApoE−/− mice (16).

ApoE is essential for the VLDL export from hepatocytes (11). In this study, both cetirizine and fexofenadine decreased the expression of ApoE mRNA in the liver. Therefore, we anticipated an increased hepatic triglyceride level by antihistamine treatments. However, we noted increased triglycerides only by fexofenadine and increased cholesterol ester by both antihistamines. It is noted that, in steatosis, not only triglycerides but also cholesterol ester, nonesterified fatty acids, ceramides, and diacylglycerols may be accumulated (8). It is probable that cetirizine and fexofenadine distinctly modulate fat accumulation in liver. In addition, fexofenadine increased serum levels of glucose, and cetirizine increased lipase. Therefore, these differences might have unique effects on steatosis. It is noteworthy that H1-antihistamines reduced serum levels of triglycerides in wild-type mice without altering HDL, LDL, and total cholesterol levels. ApoE−/− mice were completely resistant to H1-antihistamine-induced changes in triglyceride levels. Thus the decreased serum triglyceride levels in wild-type mice may be attributed to active triglyceride uptake by the liver, which is absent in ApoE−/− mice. These findings are consistent with the augmented fat deposition in the liver and adipose tissue in wild-type mice and the lack of enhanced steatosis and gonadal fat deposition in ApoE−/− mice on H1-antihistamine regimens.

To assess whether H1-antihistamine-induced increase in hepatosteatosis involves activation of lipogenic and lipid transport-associated genes, we measured their mRNA expressions.
The hepatic mRNA expression of SREBP1c, FAS, ApoB100, and LDLr were not altered by H1-antihistamines in wild-type or ApoE−/− mice. Although Wang et al. (38) also showed no change in FAS expression in H1R−/− mice, they noted a slight, but significant, increase in SREBP-1c expression. We show that H1-antihistamines downregulated the mRNA of ChREBP and ApoE in wild-type mice. It should be noted that, in patients with insulin resistance and diabetes, ChREBP expression is reduced despite the presence of steatosis (4). The significant increase in serum glucose by fexofenadine in mice indicates that fexofenadine can affect hepatosteatosis more aggressively than cetirizine.

Bile acids, produced from cholesterol, are important regulators of fat absorption, as well as glucose and lipid metabolism (6). The concentration of conjugated bile acids was significantly higher in fexofenadine-treated wild-type mice. The basolateral uptake of bile acids is mediated by Ntcp and Oatp1b2 (7). The canalicular side of hepatocytes expresses Bsep, which exports bile salts into bile (17). The increased Oatp1b2 expression, together with decreased Bsep expression, may accumulate bile acids in hepatocytes of fexofenadine-treated mice. It is also known that uptake of fexofenadine into the liver is facilitated by the Oatp family of transporters (33). Therefore, an increased uptake of fexofenadine through Oatp1b2 and its retention may be expected in liver by a mechanism similar to that of bile acid accumulation. The accumulation of bile acids in liver was less apparent in cetirizine-treated mice because Oatp1b2 was not affected by cetirizine. The concentration of unconjugated bile acids was not increased with H1-antihistamine treatments. Histamine-mediated H1R signaling is important in glucose metabolism, and H1R−/− mice are moderately insulin resistant and exhibit diabetes (38). Glucose can increase histone acetylation of the cholesterol 7α-hydroxylase (Cyp7a1) gene and induce bile acid synthesis (20). The epigenetic changes to Cyp7a1 by increased glucose in fexofenadine-treated mice may be partially responsible for increased bile acid concentration in wild-type mice.

In summary, the data presented in this report show that both cetirizine and fexofenadine worsen macrovesicular hepatosteatosis in wild-type mice, but not in ApoE−/− mice, maintained on a HFD. Furthermore, both antihistamines significantly reduce ApoE expression in the liver. Cetirizine seems to promote hepatosteatosis predominantly via lipase-mediated free fatty acid generation and cholesterol ester deposition. On the other hand, fexofenadine-enhanced hepatic steatosis involves hyperglycemia, deranged bile acid homeostasis, and triglyceride and cholesterol ester accumulation. On the basis of our findings, we have proposed a scheme (Fig. 9) depicting distinct mechanisms by which cetirizine and fexofenadine increase hepatosteatosis.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Mehrdad Maz for the critical reading of the manuscript.

GRANTS

This study was supported by NIH grants R01-HL070101 and 3R01-HL070101-04S1. Joseph and Elizabeth Carey Arthritis Fund, Audrey E. Smith Medical Research Fund from KU Endowment Association, and the Department of Internal Medicine Research Office at the University of Kansas Medical Center.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

HI-ANTIHISTAMINES ENHANCE HIGH-FAT DIET-INDUCED STEATOSIS IN MICE


