Ezetimibe ameliorates intestinal chylomicron overproduction and improves glucose tolerance in a diet-induced hamster model of insulin resistance

Mark Naples,1 Chris Baker,1 Marsel Lino,1 Jahangir Iqbal,2 M. Mahmood Hussain,2 and Khosrow Adeli1

1Molecular Structure and Function, Research Institute, The Hospital for Sick Children, University of Toronto, Toronto, Canada; 2SUNY, Downstate, New York, NY

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Ezetimibe ameliorates intestinal chylomicron overproduction and improves glucose tolerance in a diet-induced hamster model of insulin resistance. Am J Physiol Gastrointest Liver Physiol 302: G1043–G1052, 2012. First published February 16, 2012; doi:10.1152/ajpgi.00250.2011.—Ezetimibe is a cholesterol uptake inhibitor that targets the Niemann-Pick C1-like 1 cholesterol transporter. Ezetimibe treatment has been shown to cause significant decreases in plasma cholesterol levels in patients with hypercholesterolemia and familial hypercholesterolemia. A recent study in humans has shown that ezetimibe can decrease the release of atherogenetic postprandial intestinal lipoproteins. In the present study, we evaluated the mechanisms by which ezetimibe treatment can lower postprandial apoB48-containing chylomicron particles, using a hyperlipidemic and insulin-resistant hamster model fed a diet rich in fructose and fat (the FF diet) and fructose, fat, and cholesterol (the FFC diet). Male Syrian Golden hamsters were fed either Chow or FF or FFC diet ± ezetimibe for 2 wk. After 2 wk, chylomicron production was assessed following intravenous triton infusion. Tissues were then collected and analyzed for protein and mRNA content. FFC-fed hamsters treated with ezetimibe showed improved glucose tolerance, decreased fasting insulin levels, and markedly reduced circulating levels of TG and cholesterol in both the LDL and VLDL fractions. Examination of triglyceride (TG)-rich lipoprotein (TRL) fractions showed that ezetimibe treatment reduced postprandial cholesterol content in TRL lipoproteins as well as reducing apoB48 content. Although ezetimibe did not decrease TRL-TG levels in FFC hamsters, ezetimibe treatment in FF hamsters resulted in decreases in TRL-TG. Jejunal apoB48 protein expression was lower in ezetimibe-treated hamsters. Reductions in jejunal protein levels of scavenger receptor type B-1 (SRB-1) and fatty acid transport protein 4 were also observed. In addition, ezetimibe-treated hamsters showed significantly lower jejunal mRNA expression of a number of genes involved in lipid synthesis and transport, including srebplc1, sr-b1, ppar-γ, and abcg1. These data suggest that treatment with ezetimibe not only inhibits cholesteryl uptake, but may also alter intestinal function to promote improved handling of dietary lipids and reduced chylomicron production. These, in turn, promote decreases in fasting and postprandial lipid levels and improvements in glucose homeostasis.

cholesterol; intestine

OBESITY AND DYSLIPIDEMIA ARE TWO major pathophysiological conditions commonly associated with the consumption of a calorie-rich western diet, which can, over time, lead to severe health complications, including the metabolic syndrome, type 2 diabetes, and cardiovascular disease. Cholesterol overconsumption as part of the western diet has been linked to multiple pathological conditions. Addition of dietary cholesterol to a high-fat/high-fructose diet has been shown to exacerbate symptoms of the metabolic syndrome including increased fasting plasma lipids, hepatic steatosis, and insulin resistance (1). In addition, dietary cholesterol has been linked to the onset of atherosclerosis and insulin resistance (6). The pathology associated with hypercholesterolemia is caused primarily by the excess of cholesterol-enriched lipoprotein particles, including chylomicron remnants. Postprandial lipemia has been identified as one of the major risk factors for cardiovascular disease (16) and, due to their prevalence in atherosclerotic plaque formation, cholesterol-enriched chylomicron particles are thought to be a contributing factor to the development of atherosclerosis (21).

Sources of cholesterol include endogenous cholesterol synthesis, absorption of exogenous dietary cholesterol, and absorption of biliary cholesterol. Control of dietary cholesterol absorption has been shown to improve hypercholesterolemia (7, 30). Ezetimibe is a drug used for the treatment of hypercholesterolemia. Its mechanism of action is to prevent the absorption of dietary cholesterol by blocking the intestinal Niemann-Pick C1-like 1 (NPC1L1) transporter. Ezetimibe treatment has been shown to inhibit the incorporation of dietary cholesterol into lipoproteins (27), decrease diet-induced hypercholesterolemia in monkeys (31), reduce saturated fatty acid absorption (18), and reduce hyperlipidemia (20). In db/db mice, ezetimibe treatment has been shown to improve cardiac injury and vascular function as well as to improve hepatic steatosis (11). In human studies, ezetimibe treatment has been shown to improve hypercholesterolemia and decrease body weight and waist circumference (33), and to reduce hepatic TG and apoB content (4). Recent studies have indicated that the effects of ezetimibe may be more complex than would be anticipated simply by blocking intestinal NPC1L1-mediated cholesterol absorption. Ezetimibe has been shown to increase cholesterol elimination via the bile and to improve the efficiency of reverse cholesterol transport via an interaction with hepatic NPC1L1 (3). In addition, ezetimibe treatment has been linked to an increase in LDL-receptor expression (17, 24, 32) and to a decrease in LDL-cholesterol (28).

Beyond the inhibition of dietary cholesterol absorption, less is known regarding the effect of ezetimibe treatment on intestinal lipid and lipoprotein homeostasis. The intestine plays a key role in homeostasis of lipid production and metabolism (5). Previous studies have suggested that intestinal apoB48 production may be decreased with ezetimibe treatment and that treatment of CaCo2 cells with ezetimibe can decrease the expression of lipid transporters including SRB-1, NPC1L1, and ABCA1 (9). More recently, ezetimibe treatment was shown to decrease postprandial apoB48 production in addition to decreasing VLDL, LDL, and IDL cholesterol in type II diabetic
patients (2). This is an important finding, as it suggests that ezetimibe treatment can affect intestinal function and alter postprandial lipoprotein production, contributing to its beneficial effects in improving dyslipidemia, and glucose homeostasis.

In the present study, we investigated the effect of ezetimibe on postprandial lipoprotein production and the expression of proteins involved in lipid metabolism and absorption. To do this, we utilized a previously established hamster model of diet-induced insulin resistance, which has been shown to exhibit increased fasting and postprandial lipid levels coupled with impaired glucose tolerance (1). Here we demonstrate that ezetimibe not only blocks cholesterol uptake, leading to decreased chylomicron release, but also changes the expression of key genes involved in lipid transport and metabolism.

**MATERIALS AND METHODS**

**Animal protocols.** Adult male Syrian golden hamsters (*Mesocricetus auratus*) were obtained from Charles River (Montreal, QC, Canada) at a weight of 110 g. Hamsters were kept in a temperature-controlled environment on a 12:12-h light-dark cycle. After 1 wk of acclimatization, hamsters were fasted for 5 h, and anesthetized using isoflurane (3% mixed with oxygen); blood was collected via the retroorbital sinus (400 μl) to evaluate baseline plasma lipids. Hamsters were allowed free access to food and water and were fed either standard rodent chow, or a custom high-fat/high-fructose/high-cholesterol (FFC) diet (30% fat/40% fructose/25% cholesterol) supplied by Dyets (Bethlehem, PA). For ezetimibe dosing, ezetimibe was premixed with the FFC diet to achieve an approximate daily dose of 1–2 mg·kg⁻¹·day⁻¹ (FFC+EZ). Body weight and food consumption were monitored throughout the study period. Following 2 wk of feeding/dosing, hamsters were fasted for 5 h, and blood was collected via cardiac puncture for plasma lipid analysis. Tissue samples were collected, snap frozen in liquid nitrogen, and stored at −80°C for later analysis. All animal procedures were approved by the Institutional Animal Care and Use Committee at The Hospital for Sick Children.

**Plasma analyses and fast protein liquid chromatography.** Upon collection, blood was put on ice and then centrifuged at 6,000 rpm for 10 min at 4°C to isolate plasma. Plasma cholesterol, triglyceride (TG), and hepatic enzyme levels (ALT and AST) were measured using a chemical auto analyzer (model VATROS 950: Ortho-Chemical Diagnostics, Rochester, NY). Plasma insulin concentrations were determined using an ultrasensitive rat insulin ELISA (Mercodia, Uppsala, Sweden) that has previously been shown to cross-react with hamster insulin (1). To separate lipoproteins by size, 100 μl of plasma was injected into an analysis and fast protein liquid chromatography (FPLC) system and separated on an Amersham Biosciences HiLoad 16/60 Superdex 2000/10/10 column. Fractions 11–50 were collected at a rate of 0.5 ml/min, and cholesterol and TG levels were measured using commercially available colorimetric kits (Randox, Mississauga, ON, Canada).

**In vivo lipid production and isolation of triglyceride-rich lipoproteins.** To determine whether or not ezetimibe can prevent postprandial increases in intestinal chylomicron production resulting from FFC feeding, we infused Triton-W1339 (a detergent that coats and protects lipid particles from lipolysis in vivo) into hamsters given an oral fat load subsequent to 2 wk of ezetimibe treatment. Prior to the experiment, hamsters were implanted with a jugular vein catheter under isoflurane-induced general anesthesia. The catheter was exteriorized through the back of the neck and filled with heparinized saline (40 U/ml). All hamsters received 0.05 mg/kg temgesic as an postsurgical analgesic. The day after cannulation, hamsters were fasted for 8 h and baseline blood (400 μl) was collected via the jugular catheter. Hamsters then received an oral fat load of olive oil (200 μl) followed 20 min later by an intravenous infusion of Triton-W1339 (0.5 g/kg). Blood was collected via the external catheter at 30, 60, 90, 120, and 180 min after the fat load. All blood collections and infusions were performed in conscious hamsters as previously described (19). To isolate the triglyceride-rich lipoprotein (TRL) fraction, 150 μl of plasma from each Triton-infused hamster was overlaid with 4 ml of a KBr solution (1.006 g/ml density) and centrifuged for 70 min at 35,000 rpm (10°C) using an SW 55 Ti rotor (Beckman Coulter, Mississauga, ON, Canada). The TRL fraction (Svedberg flotation rate [Sf], >400) was collected as the top 300 μl of the tube.

**Intraportal glucose tolerance test.** After 12 days of dosing/feeding, hamsters were fasted for 5 h, and baseline blood glucose was measured using a handheld glucometer (Roche Diagnostics, Laval, QC, Canada) subsequent to a saphenous vein prick in conscious hamsters. Glucose was administered (2 g/kg body wt ip), and blood glucose was assessed at 15, 30, 60, and 120 min postglucose injection.

**Quantitative real-time PCR analysis.** Messenger RNA levels of various genes involved in lipid synthesis and transport [Table 1 (10, 26)] were assessed by quantitative real-time PCR (qRT-PCR). Total RNA was extracted from jejunum collected from chow, FFC, or FFC+EZ hamsters using the RNeasy Mini Plus kit (Qiagen, Mississauga, ON, Canada), and 0.4 μg of total RNA was subsequently converted to single-stranded cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Streetsville, ON, Canada). All PCR reactions were performed in quadruplicate on pooled cDNA samples using SYBR Green PCR Master Mix (Applied Biosystems) and standard RT-PCR conditions. Relative quantities of mRNA were calculated from threshold cycle (Ct) values by the comparative Ct method, using 18S rRNA as an internal reference.

**SDS-PAGE and immunoblotting.** Frozen sections of jejunum were homogenized and lysed in solubilizing buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1% Triton X-100) supplemented with protease inhibitors (Complete Mini, EDTA-free, Roche Diagnostics). Protein concentrations were determined using the Bradford method (DC Protein assay kit; Bio-Rad, Hercules, CA). Equal amounts of protein were resolved by SDS-PAGE and detected with chemiluminescent immunoblotting using the following antibodies: ATP-binding cassette subfamily G member 8 (ABCG8) and NPC1L1 from Santa Cruz Biotechnology (Santa Cruz, CA), SRB-1 from Novus Biologicals (Littleton, CO), fatty acid transport protein 4 (FATP4) from Dr. Paul Watkins (Johns Hopkins University, Baltimore, MD), and apoB from Midland BioProducts (Boone, IA).

**Microsomal triglyceride transfer protein lipid transfer activity.** One hundred milligrams of jejunum was homogenized in 1 ml of ice-cold homogenization buffer (1 mM Tris, pH 7.6, 1 mM EGTA, and 1 mM MgCl₂, and 10 μl of protease cocktail inhibitors). Samples were centrifuged at 8,000 g for 30 min at 4°C and 0.5 ml of the supernatant was extracted and flash frozen in liquid nitrogen to be used for analysis. The assay was done in Microfluor 2 Black U Bottom Microtiter plates (Thermo Labsystems, Franklin, MA). To the wells, we added 3 μl of donor (450 nmol of PC and 14 nmol of TAG per microliter), 3 μl of acceptor (2,400 nmol PC/ml) vesicle, 10 μl of 10 mM Tris-HCl buffer, pH 7.4, 2 mM EDTA, 150 mM NaCl, distilled water to make the final assay volume 100 μl and purified microsomal triglyceride transfer protein (MTP) (0.1–1.5 μg) in triplicate. Plates were incubated at 37°C and read with a fluorescence plate reader (model 7620 Microplate Fluorimeter; Cambridge Technology, Watertown, MA) using 460 nm excitation and 530-nm emission wavelengths. To determine blank values, MTP was omitted from the wells. Total fluorescence in donor vesicles was determined by adding 97 μl of isopropanol to 3 μl of donor vesicles. MTP activity (% transfer) was calculated by the following equation: % transfer = (arbitrary fluorescence units in assay wells – blank values)/(total fluorescent units – blank values) × 100. The specific activity is expressed as a % transfer per micrograms per hour.

**Statistical analyses.** Statistical significance was evaluated by one-way ANOVA followed by post hoc analysis or by using unpaired
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Table 1. Genes assessed by real-time quantitative RT-PCR

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Prime pairs for PCR were designed with Primer3 software using sequence information obtained from GenBank. *No hamster sequence was available. In these cases, primers were designed to highly conserved regions determined by multiple sequence alignments performed on closely related species.

Student’s t-tests; outcomes were deemed to be significantly different when P < 0.05.

RESULTS

Ezetimibe prevents diet-induced hyperlipidemia. Male Syrian golden hamsters were fed either the FFC diet or FFC mixed with ezetimibe (FFC+EZ; 1–2 mg·kg⁻¹·day⁻¹) for 14 days. No differences in body weight or food consumption (as determined by daily caloric intake) were observed between the FFC and FFC+EZ groups (data not shown). FFC feeding resulted in fasting hyperlipidemia: plasma TG (Fig. 1A), cholesterol (Fig. 1B), and insulin levels (Fig. 1C) all significantly increased (~2-fold) following 14 days of feeding/dosing (P < 0.05, n = 11). This dietary effect was attenuated by the inclusion of ezetimibe in the diet (TG, cholesterol, and insulin levels were all unchanged compared with baseline). We also performed fast protein liquid chromatography (FPLC) on the endpoint plasma from chow, FFC, or FFC+EZ hamsters (n = 8). Fractionation of lipoprotein particles showed that FFC feeding resulted in significant increases in VLDL-cholesterol levels compared with chow (P < 0.01) and that ezetimibe treatment prevented this increase and kept VLDL-cholesterol levels at chow-fed levels. In addition, ezetimibe treatment decreased LDL-cholesterol levels compared with FFC alone (Fig. 1E; P < 0.05). Ezetimibe treatment was also able to attenuate the increase in VLDL-TG levels seen with FFC feeding (Fig. 1D). Ezetimibe treatment also decreased LDL-TG.

Effect of ezetimibe on intestinal lipoprotein production. Upon completion of the 2-wk feeding cycle, hamsters underwent a fat absorption test to determine whether ezetimibe treatment can alter intestinal apoB48/chylomicron production. Hamsters were given an oral fat load to stimulate intestinal chylomicron production coupled with an intravenous infusion of Triton-WR1339 to protect the secreted chylomicron particles from hydrolysis. Hamsters treated with ezetimibe had decreased levels of TRL-apoB48 compared with FFC hamsters. This decrease was noticeable at 60 min and was sustained up until the 180-min endpoint (n = 3). FFC+EZ hamsters did tend to have lower TRL-apoB48 at all time points; however, this was not shown to be significant. Analysis of the slope of the apoB accumulation curve showed that there was a significantly lower accumulation of apoB48 in the TRL fraction (Fig. 2A). TRL-TG levels were essentially the same between FFC and FFC+EZ hamsters (Fig. 2B). TRL-cholesterol, however, was significantly lower at 90 and 180 min after the fat load and evaluation of the area under the curve (AUC) of the cholesterol accumulation curve revealed that TRL-cholesterol levels were significantly lower with ezetimibe treatment (Fig. 2C). In addition, the slope of the cholesterol accumulation curve indicated that ezetimibe treatment significantly lowered the
rate of TRL-cholesterol accumulation in plasma (FFC slope = 0.0053, FFC+EZ slope = 0.0039, \( P < 0.05 \)).

To determine how ezetimibe alters intestinal apoB48 secretion we evaluated the activity of MTP to determine whether the lack of apoB48 secretion was related to decreased lipidation and thus degradation of apoB48. Examination of jejunal MTP activity showed that hamsters fed FFC for 2 wk had increased MTP activity (\( \sim 30\% \)) compared with chow-fed hamsters. Treatment with ezetimibe prevented this increased activity (Fig. 2D).

Ezetimibe prevents the onset of insulin resistance. Next we evaluated the ability of ezetimibe to improve whole body insulin resistance, a characteristic feature of the FFC model (1). We performed intraperitoneal glucose tolerance tests (IPGTT) in hamsters fed FFC or FFC+EZ for 14 days (Fig. 3). IPGTT from FFC+EZ hamsters showed a significantly lower response to glucose challenge compared with FFC hamsters. Blood glucose was significantly lower in FFC+EZ hamsters at 15 and 30 min postglucose challenge compared with FFC alone (\( n = 5 \)). \( * P < 0.05 \) vs. chow-fed; \( # P < 0.05 \) vs. FFC.

Ezetimibe alters the mRNA levels of genes involved in lipid metabolism and transport. To elucidate the mechanisms whereby ezetimibe exerts its effects on lipid absorption we quantified the expression levels of several genes involved in intestinal lipid metabolism and transport (Fig. 4). Total RNA was isolated from hamsters fed chow, FFC, or FFC+EZ for 14 days, and mRNA levels were quantified by qRT-PCR (\( n = 6 \)). Compared with chow, FFC-feeding caused significant increases in jejunal srebplc (\( \sim 7\)-fold), fas (\( \sim 4\)-fold), ppar (\( \sim 3\)-fold), and abcg1 (\( \sim 3\)-fold); these increases were prevented by the inclusion of ezetimibe in the diet (with the exception of fas, which ezetimibe treatment could not normalize). In the case of abcg1, ezetimibe decreased expression levels below those observed in chow-fed hamsters, probably due to cholesterol depletion of intestinal enterocytes. Interestingly, mRNA levels for fxr were increased significantly in ezetimibe-treated hamsters compared with both chow- and FFC-fed hamsters, whereas expression of fas, lpl, and npc1l1 were all significantly increased in hamsters receiving the FFC diet compared with chow, regardless of whether or not they received ezetimibe. Additionally, we observed an increase in MTP mRNA that was significantly alleviated by ezetimibe. ApoB expression was not altered by any of the test conditions.

Ezetimibe alters the intestinal expression of lipid transport proteins. Next, we examined the effects of ezetimibe treatment on the protein levels of some of the key lipid transporters expressed in the intestine (Fig. 5). Intestinal SRB-1 (increased in FFC-fed compared with chow-fed), FATP4, and cellular apoB48 were found to be downregulated in hamsters receiving ezetimibe compared with FFC-fed controls. Protein expression of ABCG5, ABCG8, and NPC1L1 were unchanged by ezetimibe treatment. We also attempted to measure MTP protein levels in the jejunum of these hamsters; however, the blots had a great degree of variability (possibly due to antibody
cross-reactivity issues); thus these data were inconclusive (data not shown).

**Evidence for reduced apoB48 stability and increased degradation following ezetimibe treatment.** Pulse-chase experiments were also performed to assess the stability and degradation of apoB48 in enterocytes isolated from hamsters following treatment with or without ezetimibe. As shown in Fig. 6, there was a significant decline in apoB48 recovery in apoB48 treated with ezetimibe following the pulse at time 0 chase as well as some decreases at 45- and 90-min chase times, suggesting considerable loss of apoB, possibly due to cotranslational and post-translational degradation of apoB48.

**Ezetimibe regulates fasting and postprandial dyslipidemia by a mechanism that is independent of dietary cholesterol.** We next evaluated how ezetimibe regulates lipid metabolism in a model that lacks dietary cholesterol to determine whether dietary cholesterol is necessary for ezetimibe’s therapeutic effects. Hamsters were fed a diet high in fat (30%) and fructose (30%) with no cholesterol (FF), and then received either control FF diet or FF plus ezetimibe (FF + EZ) (as described above). After 2 wk of feeding, FF-fed hamsters had higher fasting plasma TG levels compared with baseline; however, unlike FFC-fed hamsters, cholesterol was unchanged with FF feeding (Fig. 7). Ezetimibe treatment in FF-fed hamsters prevented the accumulation of fasting plasma TG levels but had no effect on plasma cholesterol levels.

Chylomicron production in FF-EZ-treated hamsters was also assessed to determine the role of ezetimibe on postprandial lipid secretion. Hamsters receiving FF diet alone showed a marked increase in TRL-TG levels following an oral fat load, and this was accompanied by a similar increase in TRL apoB48 levels. Hamsters receiving ezetimibe along with FF feeding showed a significant decrease in chylomicron secretion as denoted by decreased AUC for TRL-TG (FF = 46.3 vs.
Fig. 3. Ezetimibe improves insulin resistance as assessed by intraperitoneal glucose tolerance tests (IPGTT; 2 g/kg body wt) that were performed on 5 h-fasted hamsters following 2 wk of the FFC or FFC+EZ (n = 8). AUC values are shown (inset); *P < 0.05 by 1-way ANOVA.

FF+EZ = 23.1, P < 0.05) and decreases in TRL-TG. Additionally apob48 accumulation in the TRL fraction was significantly decreased in FF+EZ hamsters as denoted by a significant decrease in the slope of the apoB accumulation curve (P < 0.05). TRL-cholesterol levels were slightly decreased with ezetimibe treatment; comparison of AUC values shows a significant difference (FF = 33.3 ± 9.8 vs. FF+EZ = 18.6 ± 8.1, P < 0.05) although there was no significant difference at any particular time point.

**DISCUSSION**

In the present study, we evaluated the effects of ezetimibe treatment on postprandial lipid production in an established model of insulin resistance and dyslipidemia. Our data suggest that ezetimibe treatment can resolve some of the negative effects of a typical western diet containing high levels of fat, fructose, and cholesterol. Ezetimibe treatment markedly reduced fasting plasma cholesterol and triglyceride to baseline levels compared with FFC-fed animals and stunted the onset of insulin resistance seen with FFC feeding. This is in agreement with previous studies that have shown ezetimibe treatment improves insulin resistance, hyperlipidemia, and hepatic steatosis in obese rats (8) and improves total cholesterol levels in humans (12). In addition to these observations, we saw a decrease in lipoprotein production with ezetimibe treatment. Ezetimibe treatment markedly reduced fasting VLDL-cholesterol accumulation in the plasma, completely masking the effects of the FFC diet. In addition ezetimibe treatment caused a significant reduction in LDL-cholesterol levels that were even lower than those seen in chow-fed animals, indicating that ezetimibe restricts cholesterol availability to the liver resulting in the formation of cholesterol poor lipoproteins. Previous studies have shown similar results, with ezetimibe treatment causing decreases in total cholesterol and LDL-cholesterol in dyslipidemic patients (12); however, this study did not show significant changes in VLDL-cholesterol accumulation. Still other studies have shown decreases in circulating levels of both VLDL- and LDL-cholesterol along with chylomicron-cholesterol in both human subjects and rat models (8, 20, 29). Mice fed a high-fat diet and receiving ezetimibe showed decreased LDL-cholesterol and TG as well as decreases in srebp-1c (22), observations that largely agree with results obtained from our hamster model and which suggest that ezetimibe treatment significantly improves plasma lipid profiles. Human trials using ezetimibe have demonstrated similar results; ezetimibe treatment was reported to improve symptoms of nonalcoholic fatty liver disease, and normalize plasma TG, total cholesterol, LDL-cholesterol, LDL-apolB, and total plasma apolB levels (4, 23, 33).

Our data suggests that ezetimibe treatment not only results in the release of cholesterol-poor chylomicron particles, but also blocks chylomicron production as evidenced by the decline in apob48 levels in the TRL fraction. The appearance of cholesterol-poor lipoprotein particles most likely stems from the lack of cholesterol availability for VLDL synthesis. Recent work by Bozzetto et al. (2) has shown similar results in humans and strongly supports the observations made here in our hamster model. Deushi et al. (8) have shown that ezetimibe decreases chylomicron-TG and cholesterol in rats, and other studies in humans have shown similar results (20, 29). Ezetimibe’s lack of effect on TRL-TG levels in FFC-fed hamsters was surprising given these findings; however, we did observe a decrease in TRL-TG from FFC-fed hamsters. Although we still do not know the contribution of the liver to postprandial TRL-TG pool in these experiments, this is most likely a result of the severity of the FFC model. The addition of cholesterol greatly worsens the phenotype of dyslipidemia and it would seem that ezetimibe cannot completely counteract these effects. Despite this, we have shown that ezetimibe can still alter postprandial lipoprotein production in a model lacking dietary cholesterol, suggesting metabolic effects beyond its inhibition of cholesterol absorption. In the present study, we also observed significantly decreased intestinal expression of sreb1 mRNA in ezetimibe-treated hamsters compared with the FFC-fed group. These changes suggest that ezetimibe treatment not only blocks cholesterol uptake by inhibiting NPC1L1 but also decreases expression of another
key cholesterol transporter (SR-B1). This indicates that, beyond inhibiting dietary cholesterol uptake, the effects of ezetimibe can extend to alter the intestine’s fundamental ability to absorb cholesterol. This indicates a compounded effect of the drug on dietary cholesterol absorption, by hindering two of the key dietary cholesterol transporters. Overall, these data present strong evidence that ezetimibe treatment can alter intestinal function beyond blocking cholesterol uptake. The alteration in expression levels of several proteins and genes previously identified to be crucial to the absorption of cholesterol and its subsequent processing into chylomicron particles shows that ezetimibe treatment can alter intestinal function toward a state

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**Fig. 5.** Lipid transporter protein expression in the jejunum of chow-, FFC-, and FFC+EZ-fed hamsters. Representative immunoblots showing jejunal expression of ABCG5, ABCG8, Niemann-Pick C1-like 1 (NPC1L1; A), and scavenger receptor type B-1 (SRB-1), fatty acid transport protein 4 (FATP4), apoB48 (B) in hamsters following 2 wk of feeding (n = 6). β-Actin was also detected as control. Each lane represents an individual animal and contains the same amount of total cellular protein.

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**Fig. 6.** Effect of ezetimibe on intracellular stability and degradation of apoB48 in hamster enterocytes. Hamsters were fed FFC diet for 2 wk with or without ezetimibe treatment. At the end of the feeding period, enterocytes were freshly isolated and used to perform pulse-chase experiments (45-min pulse, 0–90 min chase). ApoB was immunoprecipitated and visualized by SDS-PAGE/autoradiography. Data shown as mean ± SE (3 experiments, each performed in triplicate). *P < 0.05 compared with FFC.
that prevents hyperlipemia. From our results, it is apparent that ezetimibe treatment decreases postprandial chylomicron remnant release, which may explain the observed decrease in total intestinal chylomicron output. FATP4 expression was also decreased with ezetimibe treatment, indicating that the absorption of fatty acids may be hampered in these animals and that the inhibitory effects of ezetimibe on lipid absorption are not restricted to cholesterol. Previous studies have shown similar decreases in FATP4 mRNA expression (25). Labonté et al. (18) report that ezetimibe treatment decreased expression of FATP4 in intestinal scrapings, accompanied by a decrease in intestinal fatty acid absorption. The observed decrease in TRL-apoB48 release may be linked to an increase in degradation of intestinal apoB. With ezetimibe treatment, we observed a decrease in TRL-apoB48 levels that was accompanied by a decrease in fasting intestinal apoB48. Since it has been shown that apoB is degraded when there is insufficient substrate availability to form lipoprotein particles (13–15), we postulate that ezetimibe-mediated decreases in MTP activity limit substrate availability and, as such, creates a condition that favors degradation of apoB rather than packaging of lipids into chylomicron particles. In line with this hypothesis, it was observed that apoB mRNA expression was unchanged by any of the treatments; however, apoB protein levels in the intestine were decreased with ezetimibe treatment, indicating regulation of apoB48 at the protein level. There was also a considerable loss of apoB following the 45-min pulse possibly due to cotranslational degradation of apoB48. We cannot rule out a potential effect on apoB synthesis, although the lack of an effect on apoB48 mRNA levels makes it unlikely that ezetimibe reduces apoB synthesis. The significant decline in apoB48 at time 0 may be explained by increased degradation of apoB during the 45-min pulse period, as there is published evidence for cotranslational degradation of apoB polypeptides. Additionally, intestinal expression of the lipid transporters including SR-BI and FATP4 were downregulated with ezetimibe treatment, indicating that the intestine would have a decreased capacity for lipid absorption, which would limit the amount of lipid available for chylomicron assembly. The
observation that MTP activity is reduced in FFC-EZ hamsters compared with untreated animals indicates that there is less lipidation of nascent apoB48 particles, which could lead to increased degradation of newly synthesized apoB48. This would explain the decrease in chylomicron production. Our data showed that ezetimibe treatment was not sufficient to alter TRL-TG levels in FFC-fed hamsters. Given that TRL apoB48 was decreased, this would indicate that ezetimibe promotes apoB48 degradation and thus results in the production of fewer, more TG-rich chylomicron particles. Interestingly, when cholesterol is removed from the diet, there was an increased efficacy of ezetimibe, with FF-EZ hamsters showing decreased TRL-apoB48 and TG. This could be attributed to the severity of the phenotype that results from FFC feeding. We have previously shown that increasing cholesterol in a high-fat/high-fructose diet severely augments the amount of TG in both the plasma and liver. Given the observation that MTP activity is decreased in FFC-EZ hamsters, it is surprising that TRL-TG levels are not decreased, especially considering that the expression profile of intestinal lipid transporters indicates a decrease in intestinal fatty acid uptake. It is possible, however, that de novo lipogenesis in the intestine could be increased in FFC-fed hamsters and that ezetimibe treatment is not sufficient to counteract this effect. Intestinal FAS mRNA levels were increased with FFC feeding and ezetimibe treatment did not alter this. These observations indicate that ezetimibe treatment decreases MTP activity in FFC hamsters; however, ezetimibe does not counter the FFC-induced increase in FAS expression; as such there could still be increases in de novo lipogenesis accompanied by decreased lipid transfer to newly formed apoB48 proteins, this could lead to the secretion of fewer chylomicron particles. These data taken together indicate that ezetimibe may decrease dietary fatty acid absorption; however, the FFC diet promotes increased de novo lipogenesis. On the other hand, decreased availability of exogenous TG and/or decreased MTP activity may result in less rescue of apoB48 from degradation by ER lipidation. Most likely, decreased lipid availability may be the causative factor in decreased MTP activity. However, our finding that plasma TG accumulation is not affected by ezetimibe treatment (in the FFC-fed model) suggests that there is more TG per particle in the face of a reduced number of particles secreted.

Although the mechanism(s) mediating ezetimibe-induced improvement in glucose tolerance in our hamster model are unknown, our findings do support earlier observations made in rodent models. It is likely that the marked improvement in lipid homeostasis, including a significant reduction in circulating triglyceride, may contribute to improved whole body insulin sensitivity. There may also be a direct effect of ezetimibe on intestinal, and possibly hepatic, insulin signaling, which contributes to increased glucose tolerance. There is now considerable evidence for ezetimibe-induced amelioration of hepatic steatosis in obese humans (4) and fat-fed mice (11). Reductions in hepatic fat may underlie the observed improvements in whole body insulin sensitivity.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.N., C.B., M.L., J.J., and M.M.H. performed experiments; M.N., C.B., and K.A. analyzed data; M.N., C.B., and K.A. interpreted results of experiments; M.N. and C.B. prepared figures; M.N. and C.B. drafted manuscript; K.A. conception and design of research; K.A. edited and revised manuscript; K.A. approved final version of manuscript.

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