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Antisense inhibition of apoB synthesis with mipomersen reduces plasma apoC-III and apoC-III-containing lipoproteins

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Abstract **Mipomersen, an antisense oligonucleotide that reduces hepatic production of apoB, has been shown in phase 2 studies to decrease plasma apoB, LDL cholesterol (LDL-C), and triglycerides. ApoC-III inhibits VLDL and LDL clearance, and it stimulates inflammatory responses in vascular cells. Concentrations of VLDL or LDL with apoC-III independently predict cardiovascular disease. We performed an exploratory posthoc analysis on a subset of hypercholesterolemic subjects obtained from a randomized controlled dose-ranging phase 2 study of mipomersen receiving 100, 200, or 300 mg/wk, or placebo for 13 wk (n = 8 each). ApoC-III–containing lipoproteins were isolated by immuno-affinity chromatography and ultracentrifugation. Mipomersen 200 and 300 mg/wk reduced total apoC-III from baseline by 6 mg/dl (38–42%) compared with placebo group ($P < 0.01$), and it reduced apoC-III in both apoB lipoproteins and HDL. Mipomersen 100, 200, and 300 mg doses reduced apoB concentration of LDL with apoC-III (27%, 38%, and 46%; $P < 0.05$). Mipomersen reduced apoC-III concentration in HDL. The drug had no effect on apoE concentration in total plasma and in apoB lipoproteins. In summary, antisense inhibition of apoB synthesis reduced plasma concentrations of apoC-III and apoC-III–containing lipoproteins. Lower concentrations of apoC-III and LDL with apoC-III are associated with reduced risk of coronary heart disease (CHD) in epidemiologic studies independent of traditional risk factors.**—Furtado, J. D., M. K. Wedel, and F. M. Sacks. **Antisense inhibition of apoB synthesis with mipomersen reduces plasma apoC-III- and apoC-III-containing lipoproteins.** ***J. Lipid Res.*** **2012.** 53: **784–791.**

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Apolipoprotein (apo)C-III is a protein present on some apoB lipoproteins that is not integral to the lipoprotein structure but that modifies the metabolism in plasma of the lipoprotein particle. Concentration of apoC-III in very low- and low-density lipoproteins (VLDL and LDL) is highly and independently predictive of coronary heart disease (CHD), more so than triglyceride alone (1). LDL with apoC-III, a remnant particle produced by partial lipolysis in plasma of VLDL with apoC-III (2), is the lipoprotein particle type most predictive for cardiovascular disease in type 2 diabetes (3) and in the general population (4). ApoC-III blocks the clearance of VLDL by the liver (5–7), channeling it to formation of LDL (2, 4, 7), increases adhesion of human monocytes to endothelial cells (8), and activates pro-inflammatory molecules, such as nuclear factor κB (NFκB), in these cells (8–10). ApoC-III on HDL lipoproteins may result in a dysfunctional subpopulation (8) that is associated with increased CHD (1, 11, 12).

Mipomersen is a second-generation 20-mer antisense oligonucleotide that reduces hepatic production of apoB-100. Animal models showed its potential for cardiovascular benefits through reduction of VLDL and LDL (13), as well as reduction of lipoprotein (a) and oxidized phospholipids levels (14). Mipomersen dose-dependently reduces plasma total apoB and LDL cholesterol (LDL-C), and it reduces plasma non-HDL cholesterol (HDL-C) and triglycerides in polygenic and familial hypercholesterolemia (15, 16) and in subjects with mild to moderate hyperlipidemia (17). There are no effects on plasma HDL-C concentration.

We hypothesize that mipomersen reduces the plasma concentration of apoC-III and the concentration of apoB lipoproteins that contain apoC-III. This could occur if reduced apoB synthesis decreases the amount of VLDL to which apoC-III can attach in the hepatocyte before its secretion into the circulation. It is also possible that reduced hepatic apoB content reduces apoC-III synthesis.

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Abbreviations: CHD, coronary heart disease; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NFκB, nuclear factor κB.

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MATERIALS AND METHODS

Participants

This exploratory analysis was performed on a subset of samples from a randomized, double-blind, placebo-controlled dose-escalation Phase II study (registered as NCT00216463 at ClinicalTrials.gov) in which mipomersen was evaluated as a monotherapy in subjects with primary hypercholesterolemia. The primary aim of the parent study was to determine the effects of mipomersen on concentrations of LDL-C and apoB. Recruitment protocol, drug administration, and results have been published (17). Briefly, the parent study enrolled 50 hypercholesterolemic men and women between the ages of 18 and 65 years. Inclusion criteria were a body mass index (BMI) between 25 and 32 kg/m^2 inclusive, fasting LDL-cholesterol greater than or equal to 130 mg/dl (3.36 mmol/l), and triglycerides less than or equal to 400 mg/dl (4.55 mmol/l).Women with childbearing potential were excluded. Subjects were healthy with no medical conditions or clinical abnormalities. Subjects could not take concomitant medications within 14 days of dosing (except hormone replacement therapy), use aspirin or acetaminophen for more than 5 consecutive days, or any lipid-lowering drug within 30 days or five half-lives (of the lipid-lowering drug), whichever was longer, prior to screening. Participation occurred from August 2005 to December 2006. Written informed consent was obtained prior to enrollment.

Participants were randomly assigned among five groups, having effective weekly doses of 50, 100, 200, 300, and 400 mg. Each group had 10 subjects, 8 receiving mipomersen and 2 receiving placebo. The duration of administration was 13 weeks. Three of these groups (100, 200 and 300 mg) were included in this substudy on apoC-III. In the 100 mg group, mipomersen or placebo was administered as a 200 mg subcutaneous injection in four loading doses within the first 11 days, followed by a 200 mg subcutaneous injection every other week for 11 weeks. This dosing resulted in an effectual 100 mg/wk dose because of the long plasma terminal elimination half-life of mipomersen of 30 days (18). In the 200 mg and 300 mg groups, mipomersen or placebo was administered once per week for 13 weeks. For the purposes of this study, the 2 participants receiving placebo from the mipomersen 400 mg group were also included to produce a balanced analysis of 8 participants, each of whom received placebo, 100 mg/wk, 200 mg/wk, or 300 mg/wk.

Baseline blood samples were collected on day 4 for the 100 mg group and on day 8 for the 200 mg and 300 mg groups rather than before the first dose because the predose baseline sample was not available for this study. Previous studies have shown no changes in lipids and lipoproteins within the first week after the first dose (15). Posttreatment blood samples were collected eight days after the final dose on day 99, except for one participant in the 100 mg group who left the study early at day 50 and one participant in the 300 mg group who terminated at day 22. Both of these participants were mipomersen recipients. Plasma samples were stored at −70°C and shipped to the lab in a single batch on dry ice. The analysis was conducted over the course of two months in June and July 2008 in five analysis batches. To minimize assay bias, batches consisted of 14 or 16 samples arranged to ensure that all treatment arms were present in each analysis batch and that each participant's pre- and posttreatment samples were present in the same batch. All lab staff was blinded to the treatment arm, whether the sample was pre- or posttreatment, and whether the participant was given mipomersen or placebo.

The protocol complied with the requirements of the European Clinical Trial Directive 2001/20/EC, applicable German Drug Law, and the revised Declaration of Helsinki (Washington, 2002). The study was reviewed and approved by the local institutional review board at Harvard School of Public Health. Drug safety was monitored with clinical laboratory evaluations, physical examinations, 12-lead electrocardiograms, and vital signs. Follow-up was conducted for six months posttreatment.

Immuno-affinity chromatography

Samples were removed from cryogenic storage and thawed in the dark at room temperature for 30 min. Samples were filtered and 50 mcl was reserved for whole plasma analysis. The remaining filtered plasma was recorded (volume range 0.151–0.723 ml) and loaded into 20 ml Econo-Pac columns (Bio-Rad Laboratories, Hercules, CA) packed with 2.5 ml anti-apoC-III resin (polyclonal goat anti-human apoC-III antibody bound to Sepharose 4B resin (Academy Biomedical Co., Houston, TX) at a minimum concentration of 5 mg of antibody per 1 ml of resin. The highest concentration of plasma apoC-III found in this study was ∼26 mg/dl (0.26 mg/ml). At a load volume of 0.7 ml, this is a maximum load of 0.18 mg, which is below the minimum theoretical capacity of 0.644 mg of apoC-III based on column specifications. The antibody used in the resin was purified using immuno-affinity chromatography incorporating human apoC-III bound to Sepharose resin. We have tested this antibody for cross-reactivity with other apolipoproteins (apoB, apoE, and apoAI) and found no interaction. Prior to starting this study, we tested the column resin efficiency and found that 99 ± 1% of recovered apoC-III was found in the bound fraction. Lab control samples run in each batch alongside the unknowns showed a column efficiency of 97 ± 2%. Recovery in these lab controls assessed by cholesterol concentration was 96 ± 9%. Samples and resin were incubated for 16 h at 4°C with mixing. The unbound fraction was eluted from the column by gravity followed by washes with phosphate-buffered saline (PBS). The bound fraction was then eluted from the columns with 3M sodium thiocyanate in PBS and was immediately desalted by multiple rinses in Vivaspin 20 ultrafiltration centrifugal device with PES membrane at 50,000 MWCO (Sartorius Stedim Biotech S.A., Aubagne, France), ending with a final sample volume of 700 μl.

Ultracentrifugation

The bound and unbound fractions were then further separated into density fractions by ultracentrifugation. VLDL was isolated by overlaying the 700 μl of sample with 300 μl of d = 1.006 g/ml PBS solution (OmniPur; EMD, Darmstadt, Germany) and spinning for 16 h at 15°C and 25,000 rpm in the outer-most row of a Beckman 25-Ti rotor with a Beckman L8-70M ultracentrifuge (Beckman Coulter, Inc., Fullerton, CA). The top 200 ± 10 μl from each tube was collected by careful aspiration and briefly stored at 4°C pending same-day analysis of lipids and apolipoproteins while the next ultracentrifugation step for LDL was prepared. LDL was isolated by increasing the density of the plasma remaining after VLDL aspiration with KBr solution to produce a solute density of 1.063 g/ml, and then spinning for 24 h under the same conditions as for VLDL isolation. The top 300 ± 10 μl from each was collected by aspiration. Three density fractions of plasma were thus isolated: $d < 1.006$ g/ml (VLDL), 1.006 g/ml $< d <$ 1.063 g/ml (LDL), and $d > 1.063$ g/ml (HDL). The $d > 1.063$ fraction was treated with dextran sulfate and magnesium chloride to remove any residual apoB lipoproteins, and then was desalted. The products of the combined immuno-affinity chromatography and UC density fractionation were: VLDL without apoC-III, VLDL with apoC-III, LDL without apoC-III, LDL with apoC-III, HDL without apoC-III, and HDL with apoC-III.

Determination of lipids and apolipoproteins

Sandwich ELISA procedures using affinity purified antibodies (Academy Biomedical Co.) were performed to determine concentrations of apoB, apoC-III, and apoE in whole plasma and the lipoprotein fractions. Cholesterol was determined enzymatically (Thermo Scientific, Waltham, MA). Liquid transfer for 96-well plate loading and ELISA dilutions were handled robotically with a Multiprobe II (PerkinElmer, Waltham, MA). Both ELISA and lipids plates were read with a BioTek ELx808iu 96-well plate reader controlled by KCJunior software (BioTek, Winooski, VT). All assays were completed in triplicate, and any sample with an intra-assay coefficient of variation over 15% was repeated. Final data were exported to Microsoft Excel for analysis and database management.

Statistical analyses

The primary measurements of interest were the concentrations of apoC-III in whole plasma, in apoB lipoproteins, and in HDL; and apoB concentrations in whole plasma and in VLDL and LDL containing apoC-III. Concentrations of apoB in subfractions without apoC-III, as well as apoE and cholesterol concentrations, were also studied. Effect of treatment on these lipids and apolipoproteins was calculated by subtracting baseline concentration from concentration at end of treatment. The placebo-adjusted effects were calculated as changes in participants who received placebo subtracted from the changes in those who received mipomersen in each arm to compute the effective mipomersen treatment. All statistical tests were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Differences between baseline and posttreatment concentrations within each treatment group were tested for significance using paired *t*-test. We used generalized linear models (SAS PROC GLM) to compare changes from baseline in the treatment groups with the changes from baseline in the placebo group, to test whether changes from baseline in the treatment and placebo groups were significantly different from zero, and to test whether placebo-adjusted changes from baseline in the treatment groups were significantly different from zero (defined as $P < 0.05$). Tests for trend across doses were performed by linear regression (SAS PROC REG), where change in lipoprotein concentration was the dependent variable and dose was the independent variable with values of 100, 200, and 300. We did not adjust *P* values for multiple comparisons. Sample data were excluded from statistical analysis if laboratory measurements of baseline or posttreatment samples were extreme outliers or too low to be measured accurately. Two participants in the 300 mg dosage group were excluded due to insufficient sample volume available to allow for detection of apoC-III in plasma fractions. The actual sample size for each statistical analysis is indicated in **Tables 1–4**.

RESULTS

ApoC-III

Mipomersen 200 mg/wk and 300 mg/wk reduced total plasma apoC-III by 4.2 and 4.4 mg/dl (Table 1). The net change in total apoC-III after subtracting the change in the placebo group is −6 mg/dl for both doses ($P < 0.01$; **Fig. 1**), a reduction of 42% and 38% ($P < 0.03$). These same doses resulted in placebo-adjusted reductions of apoC-III concentration in apoB lipoproteins of 2.2 and 2.1 mg/dl (37% and 35%) and in apoAI lipoproteins (HDL) of 3.7 and 5.6 mg/dl (45% and 51%, $P < 0.005$). The test for trend across doses was significant for total apoC-III and apoC-III in HDL (both $P < 0.03$).

ApoB lipoproteins with apoC-III

Mipomersen 100 mg, 200 mg, and 300 mg doses lowered the apoB concentration of LDL with apoC-III similarly by 3.2, 3.5, and 2.2 mg/dl, respectively ($P < 0.04$) (2.5, 2.7, and 1.4 mg/dl adjusted for placebo) (Table 2). These equate to dose-dependent reductions of 35–69%, adjusting for dose group–specific baseline values (*P* for trend = 0.05; **Fig. 2**). The 300 mg/wk dose reduced apoB in VLDL with apoC-III by 0.101 mg/dl (54%), and there was a significant dose-response trend across the treatment groups. The 300 mg/wk dose reduced the cholesterol concentration

TABLE 1. Effect of mipomersen treatment on apoC-III concentrations (mg/dl)

	Placebo	100 mg/wk	200 mg/wk	300 mg/wk
Total				
N	8	8	8	6[a]
Mean baseline (SD)	16.2 (2.8)	11.7 (4.4)	13.7 (5.3)	16.1 (5.9)
Mean change (SD)	1.61 (2.16)	2.58 (4.93)	−4.19 (5.19)[c]	−4.42 (2.71)[c]
P value[b]		0.6	0.01	<0.001
Placebo-adjusted mean change (SD)		0.961 (4.93)	−5.81 (5.19)[c]	−6.03 (2.71)[c]
In apoB lipoproteins				
N	8	5[a]	7[a]	5[a]
Mean baseline (SD)	8.17 (3.93)	4.38 (1.55)	5.86 (3.56)	5.87 (1.79)
Mean change (SD)	−0.239 (2.07)	0.029 (3.19)	−2.41 (2.93)	−2.29 (1.68)
P value[b]		0.9	0.1	0.09
Placebo-adjusted mean change (SD)		0.268 (3.19)	−2.17 (2.93)[c]	−2.06 (1.68)
In apoAI lipoproteins				
N	8	8	8	6[a]
Mean baseline (SD)	8.06 (3.87)	6.93 (2.78)	8.21 (4.30)	11.1 (6.03)
Mean change (SD)	1.85 (3.11)	1.75 (2.67)	−1.88 (4.47)	−3.75 (6.91)[c]
P value[b]		0.9	0.07	0.06
Placebo-adjusted mean change (SD)		−0.106 (2.67)	−3.73 (4.47)[c]	−5.60 (6.9)[c]

[a]Observations were excluded from analysis if either baseline or posttreatment observations were extreme outliers.

[b]*P* value comparing treatment mean change from baseline to placebo mean change from baseline by generalized linear model (SAS PROC GLM).

[c]*P* value for change from baseline is <0.05 by generalized linear model (SAS PROC GLM).

TABLE 2. Effect of mipomersen treatment on apolipoprotein B concentrations (mg/dl)

	Placebo	100 mg/wk	200 mg/wk	300 mg/wk
Total				
N	8	8	8	8
Mean baseline (SD)	131 (18)	131 (28)	125 (21)	137 (18)
Mean change (SD)	1.38 (16.6)	−28.9 (12.9)[c]	−55.9 (10.8)[c]	−83.1 (12.6)[c]
P value[b]		0.001	<0.001	<0.001
Placebo-adjusted mean change (SD)		−30.3 (12.9)[c]	−57.3 (10.8)[c]	−84.5 (12.6)[c]
In VLDL with apoC-III				
N	7[a]	6[a]	6[a]	5[a]
Mean baseline (SD)	0.382 (0.503)	0.152 (0.159)	0.093 (0.100)	0.187 (0.138)
Mean change (SD)	−0.088 (0.204)	0.069 (0.122)	−0.022 (0.059)	−0.101 (0.096)[c]
P value[b]		0.05	0.4	0.9
Placebo-adjusted mean change (SD)		0.157 (0.122)[c]	0.066 (0.059)	−0.013 (0.096)
In LDL with apoC-III				
N	8	7[a]	6[a]	8
Mean baseline (SD)	8.31 (3.92)	9.00 (4.14)	7.23 (2.50)	3.14 (2.13)
Mean change (SD)	−0.721 (3.15)	−3.19 (3.15)[c]	−3.46 (2.65)[c]	−2.16 (1.78)[c]
P value[b]		0.1	0.1	0.4
Placebo-adjusted mean change (SD)		−2.47 (3.15)[c]	−2.74 (2.65)[c]	−1.44 (1.78)[c]
In VLDL without apoC-III				
N	8	7[a]	8	8
Mean baseline (SD)	2.16 (1.20)	1.98 (1.85)	1.77 (1.26)	2.14 (0.81)
Mean change (SD)	0.543 (1.15)	−1.01 (1.86)[c]	−0.316 (0.787)	−0.953 (1.13)[c]
P value[b]		0.03	0.2	0.03
Placebo-adjusted mean change (SD)		−1.55 (1.86)[c]	−0.859 (0.787)[c]	−1.50 (1.13)[c]
In LDL without apoC-III				
N	8	8	8	8
Mean baseline (SD)	120 (14)	121 (27)	117 (20)	131 (17)
Mean change (SD)	1.568 (15.3)	−26.4 (12.4)[c]	−53.8 (10.0)[c]	−79.9 (12.6)[c]
P value[b]		0.001	<0.001	<0.001
Placebo-adjusted mean change (SD)		−27.9 (12.3)[c]	−55.4 (10.0)[c]	−81.4 (12.6)[c]

[a]Observations were excluded from analysis if either baseline or posttreatment observations were extreme outliers.

[b]*P* value comparing treatment mean change from baseline to placebo mean change from baseline by generalized linear model (SAS PROC GLM).

[c]*P* value for change from baseline is <0.05 by generalized linear model (SAS PROC GLM).

in VLDL with apoC-III by 0.8 mg/dl (63%) adjusted for placebo ($P = 0.001$) and in LDL with apoC-III by 1.4 mg/dl (21%) (Table 3). There were no significant changes in apoE concentration in whole plasma or in the lipoprotein subfractions with apoC-III (Table 4). However, significant reductions in apoB with no change in apoE resulted in enrichment of apoB lipoproteins with apoE after the 300 mg/wk dose (**Fig. 3**).

ApoB lipoproteins without apoC-III

Concentration of both VLDL without apoC-III and LDL without apoC-III were also reduced in a dose-response manner (Fig. 2). All three doses reduced the concentration of VLDL without apoC-III similarly by 0.9 to 1.6 mg/dl (all $P < 0.05$; Table 2), a placebo-adjusted percentage difference of 57% to 87% (P for trend = 0.03). The three doses of mipomersen reduced apoB in LDL without apoC-III in a dose-dependent manner by 28, 55, and 81 mg/dl, respectively (all $P < 0.0001$). There was a dose-response reduction in cholesterol concentration of both VLDL without apoC-III (2.5, 4.7, and 5.7 mg/dl, all $P < 0.05$, P for trend < 0.05) and LDL without apoC-III (25, 56, and 96 mg/dl; all $P < 0.05$, P for trend < 0.0001; Table 3). As in the apoB lipoproteins with apoC-III, there were no changes in apoE concentration in the apoB lipoproteins without apoC-III (Table 4). However, significant reductions in apoB with no change in apoE resulted in enrichment of apoB lipoproteins with apoE after the 200 and 300 mg/wk doses (Fig. 3). Mipomersen reduced total apoB in a dose-response manner by 30, 57, and 85 mg/dl with 100, 200, and 300 mg doses (all $P < 0.001$ compared with placebo; P for trend <0.0001; Table 2 and Fig. 2).

DISCUSSION

It has been previously reported that mipomersen diminishes synthesis of apoB in the liver, dose-dependently reducing plasma total apoB, LDL-C, non–HDL-C, and triglycerides in polygenic and familial hypercholesterolemia (15, 16), as well as in subjects with mild to moderate hyperlipidemia (17). In this study, we asked whether mipomersen reduces apolipoprotein concentrations of apoC-III and apoE. We now report that apoC-III concentration in both apoB and apoAI lipoproteins, as well as the concentration of apoB lipoproteins that contain apoC-III, are all reduced by mipomersen, whereas there is no effect on apoE concentration. Taken together, these are modifications that result in a less atherogenic lipoprotein profile.

Reduced apoC-III concentration on lipoproteins enhances recognition by hepatic receptors involved in the uptake of VLDL and intermediate-density lipoproteins (IDL) (19), directing these lipoproteins to clearance and away from lipolysis, thus reducing the concentration of highly atherogenic LDL particles with apoC-III (7).

TABLE 3. Effect of mipomersen treatment on cholesterol concentrations (mg/dl)

	Placebo	100 mg/wk	200 mg/wk	300 mg/wk
Total				
N	8	8	8	8
Mean baseline (SD)	248 (36)	252 (44)	245 (26)	244 (30)
Mean change (SD)	−4.03 (33.2)	−37.3 (14.1)[c]	−82.4 (14.0)[c]	−113 (16)[c]
P value[b]		0.004	<0.001	<0.001
Placebo-adjusted mean change (SD)		−33.3 (14.1)[c]	−78.4 (14.0)[c]	−108.5 (15.8)[c]
In VLDL with apoC-III				
N	8	8	7[a]	7[a]
mean baseline (SD)	1.09 (0.91)	0.682 (0.176)	0.658 (0.189)	1.21 (1.13)
mean change (SD)	0.340 (0.613)	−0.015 (0.383)	0.183 (0.555)	−0.427 (0.392)
P value[b]		0.2	0.3	0.03
Placebo-adjusted mean change (SD)		−0.355 (0.383)	−0.157 (0.555)	−0.767 (0.392)[c]
In LDL with apoC-III				
N	8	8	8	8
Mean baseline (SD)	16.6 (6.4)	13.0 (3.31)	13.6 (6.72)	6.78 (3.80)
Mean change (SD)	−2.29 (5.36)	−3.88 (4.36)[c]	−3.44 (6.98)	−3.69 (3.35)[c]
P value[b]		0.6	0.7	0.6
Placebo-adjusted mean change (SD)		−1.58 (4.36)	−1.15 (6.98)	−1.39 (3.35)
In VLDL without apoC-III				
N	8	7[a]	8	8
Mean baseline (SD)	9.57 (4.21)	5.27 (3.48)	7.66 (4.29)	9.93 (3.29)
Mean change (SD)	2.13 (3.06)	−0.372 (2.66)	−2.52 (1.84)[c]	−3.56 (3.76)[c]
P value[b]		0.1	0.004	<0.001
Placebo-adjusted mean change (SD)		−2.50 (2.66)[c]	−4.65 (1.84)[c]	−5.69 (3.76)[c]
In LDL without apoC-III				
N	8	8	8	8
Mean baseline (SD)	157 (28)	158 (50)	161 (23)	177 (27)
Mean change (SD)	−11.4 (28.9)	−35.8 (14.1)[c]	−67.0 (14.1)[c]	−107 (16.4)[c]
P value[b]		0.02	<0.001	<0.001
Placebo-adjusted mean change (SD)		−24.5 (14.1)[c]	−55.7 (14.1)[c]	−95.7 (16.4)[c]

[a]Observations were excluded from analysis if either baseline or posttreatment observations were extreme outliers.

[b]*P* value comparing treatment mean change from baseline to placebo mean change from baseline by generalized linear model (SAS PROC GLM).

[c]*P* value for change from baseline is <0.05 by generalized linear model (SAS PROC GLM).

ApoC-III itself activates adhesion molecules and the inflammatory transcription factors protein kinase C (PKC) and NFκB in endothelial cells and monocytes (8–10), crucial steps toward the recruitment of monocytes into the artery wall and formation of foam cells and atherosclerotic plaques. Thus, reductions in apoC-III concentration of lipoproteins as well as fewer lipoproteins containing apoC-III could result in diminished atherosclerotic plaque

TABLE 4. Effect of mipomersen treatment on apolipoprotein E concentrations (mg/dl)

	Placebo	100 mg/wk	200 mg/wk	300 mg/wk
Total				
N	8	8	8	8
Mean baseline (SD)	9.11 (3.14)	7.00 (2.86)	8.12 (2.65)	6.44 (1.93)
Mean change (SD)	−0.074 (1.25)	0.676 (2.07)	−1.61 (1.58)	−1.15 (1.53)
P value[b]		0.4	0.07	0.2
Placebo-adjusted mean change (SD)		0.750 (2.07)	−1.53 (1.58)	−1.07 (1.53)
In apoB lipoproteins with apoC-III				
N	8	7[a]	7[a]	7[a]
Mean baseline (SD)	0.859 (0.915)	0.317 (0.318)	0.475 (0.657)	0.496 (0.471)
Mean change (SD)	−0.108 (0.722)	−0.074 (0.305)	−0.014 (0.809)	−0.248 (0.433)
P value[b]		0.9	0.8	0.7
Placebo-adjusted mean change (SD)		0.034 (0.304)	0.094 (0.809)	−0.140 (0.433)
In apoB lipoproteins without apoC-III				
N	8	8	8	7[a]
Mean baseline (SD)	1.97 (0.93)	1.58 (0.97)	1.91 (1.17)	1.36 (0.55)
Mean change (SD)	−0.046 (0.540)	0.292 (0.388)	−0.020 (1.24)	−0.184 (0.605)
P value[b]		0.4	0.9	0.7
Placebo-adjusted mean change (SD)		0.338 (0.388)	0.026 (1.24)	−0.138 (0.605)

Mean changes from baseline and placebo-adjusted changes from baseline were not significant ($p > 0.05$) by generalized linear model (SAS PROC GLM).

[a]Observations were excluded from analysis if either baseline or posttreatment observations were extreme outliers.

[b]*P* value comparing treatment mean change from baseline to placebo mean change from baseline by generalized linear model (SAS PROC GLM).

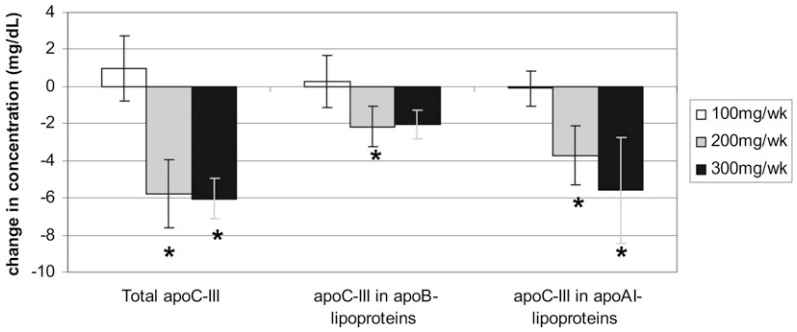

Fig. 1. ApoC-III in total plasma, apoB lipoproteins, and apoAI lipoproteins. Placebo-adjusted change (mg/dl ± SEM) *$P < 0.05$ in generalized linear model relating outcome measure to dose variable (SAS PROC GLM). P value for test for trend across doses is <0.05 for total apoC-III and apoC-III in apoAI lipoproteins.

formation and progression. Epidemiologic studies corroborate this: LDL with apoC-III is the lipoprotein particle type most predictive for cardiovascular disease in type 2 diabetes (3). And in a study of 739 healthy men and women, the risk of CHD associated with LDL with apoC-III concentration was greater than that of LDL without apoC-III, even when included together in the statistical models, suggesting that the risk of CHD contributed by LDL results to a large extent from LDL that contains apoC-III (20). Additionally, a genome-wide association study found that heterozygous carriers of a null mutation (R19×) in the apoC-III gene expressed half the amount of apoC-III present in noncarriers and had lower fasting and postprandial serum triglycerides, higher levels of HDL-C, lower levels of LDL-C, and less coronary artery calcification, which indicates that lifelong deficiency of apoC-III has a cardioprotective effect (21). This study suggests that apoC-III may have a causal role in promoting atherosclerosis and coronary disease events; therefore, reduction of apoC-III by mipomersen should produce cardioprotective effects independent of reductions in apoB.

The mipomersen-induced reduction we found in apoC-III concentration in apoB lipoproteins was in approximate proportion to the reduction in apoB concentration. Although the inhibitory effect of mipomersen on apoB synthesis appears to result in less apoC-III released from the liver, it is highly unlikely that this is due to a direct effect of mipomersen on apoC-III synthesis. Mipomersen was synthesized to be specifically complementary to the sequence of human apoB mRNA, and the mRNA sequence for apoC-III lacks complementary binding regions. Mipomersen has been shown to not bind to murine apoB mRNA (14), which also lacks complementary binding regions. In fact, species-specific apoB antisense inhibitors had to be created for testing in multiple animals species, including mice, hamsters, rabbits, and monkeys (18). We cannot, however, rule out indirect effects on apoC-III synthesis through decreases in apoB synthesis and thus cannot tell whether less apoC-III is produced in the hepatocyte or whether the same amount is produced but less is secreted. For example, it is possible that reduced VLDL formation in the hepatocyte could downregulate apoC-III synthesis. Alternatively, fewer apoB lipoproteins available to carry the apoC-III out of the hepatocytes into circulation could lead to unutilized apoC-III in the hepatocytes, reducing transcription of the apoC-III gene or increasing its posttranscriptional degradation. Although apoE is also secreted from the liver as part of the lipoprotein complex, we found no effect of mipomersen on apoE. If apoC-III and apoE secretion are regulated solely by the amount of apoB available to carry them out into circulation, then both apoC-III and apoE concentrations would be reduced when apoB secretion is reduced. These data suggest that there may be different mechanisms in the regulation of secretion of apoC-III and apoE as components of apoB lipoproteins.

ApoC-III in HDL was reduced by the 200 and 300 mg/wk doses. A direct effect from mipomersen on HDL is not likely because the basic structure of this lipoprotein class is formed of apoAI, not apoB. However, apoC-III is transferred from triglyceride-rich lipoproteins to HDL during lipolysis (22), so reductions in the secretion of apoC-III mediated by reductions in apoB could plausibly result in downstream reductions in HDL apoC-III. The clinical effect of this is not clear, although there are studies suggesting that apoC-III may alter the function of HDL or its relation to CHD (1, 11). For example, HDL with apoC-III did not reduce adhesion of human monocytic cells to endothelial cells as HDL without apoC-III did, suggesting that apoC-III may have counteracted the

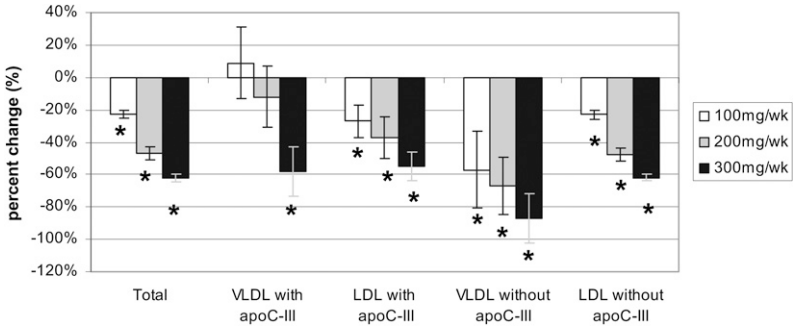

Fig. 2. ApoB in total plasma, and VLDL and LDL with and without apoC-III. Placebo-adjusted change (mg/dl ± SEM). *$P < 0.05$ in generalized linear model relating outcome measure to dose variable (SAS PROC GLM). P value for test for trend across doses is <0.05 for total plasma and all plasma fractions.

protective actions of HDL (9). In a study comparing 18 men with established coronary artery disease (CAD) with 20 apparently healthy men, apoC-III concentration in HDL_2 was higher in CAD subjects than in the healthy controls (12).

Although there were no changes in apoE concentration related to mipomersen treatment, we found that the large reductions in apoB with no change in apoE results, on average, in enrichment of apoB lipoproteins with apoE (after the 300 mg/wk dose for those also containing apoC-III and after both the 200 and 300 mg/wk doses for those without apoC-III; Fig. 3). Given that apoE is recognized by hepatic receptors, this apoE enrichment could result in a greater clearance of apoB lipoproteins from circulation (23, 24). Kinetic studies of apoB metabolism showed that apoB lipoproteins with both apoC-III and apoE compared with those with apoC-III and no apoE are directed away from lipolysis that produces LDL toward clearance from the circulation (7). If this enrichment of apoE results in more apoB lipoproteins with both apoC-III and apoE rather than with apoC-III alone, formation of atherogenic remnants and LDL could be reduced. However, this speculation needs support from measurement of the concentration of apoB lipoproteins with apoE on them in addition to the concentration of apoE on these apoB lipoproteins.

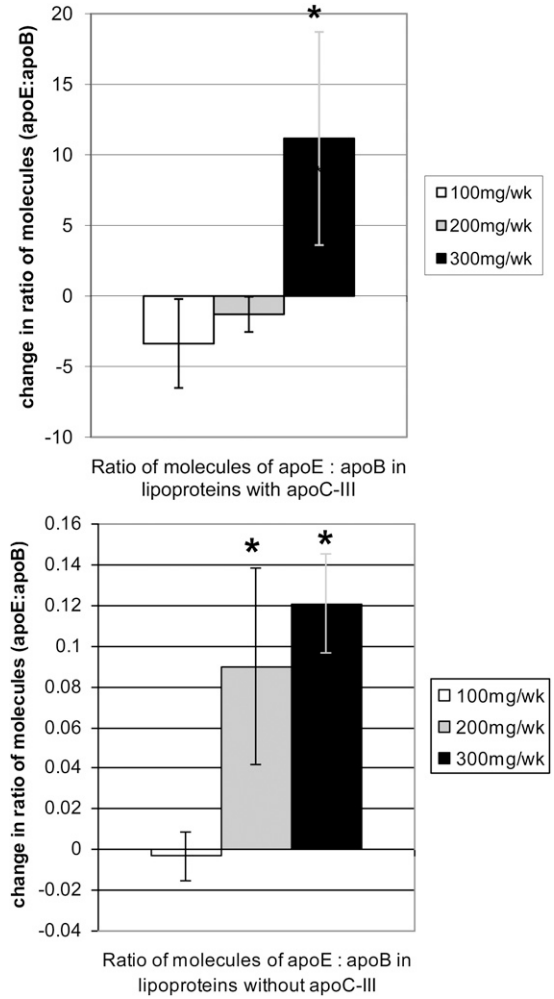

Fig. 3. ApoE per apoB ratio of molecules (± SEM) in lipoproteins with and without apoC-III. $*P < 0.05$ in generalized linear model relating outcome measure to dose variable (SAS PROC GLM). *P* value for test for trend across doses is <0.05 for both.

Although the sample size of this study is more than sufficient for strength of effect of mipomersen on plasma total apoB and cholesterol, it would have been desirable to have a larger sample size for the less abundant subfractions, such as VLDL with apoC-III. The concentration of VLDL with apoC-III is lower in this study than in our previous studies using the same methods. It is possible that this VLDL component underwent degradation during storage. The other lipoprotein types had concentrations similar to previous reports.

CONCLUSION

We have found that mipomersen reduces total apoC-III, apoC-III in apoB lipoproteins, and apoC-III in HDL. ApoC-III is an activator of atherogenic processes, and apoB lipoproteins with apoC-III are associated with increased CHD. Mipomersen has no effect on apoE concentrations, thereby enriching apoB lipoproteins with apoE relative to apoC-III, which may facilitate lipoprotein clearance and reduce formation of atherogenic remnants and LDL.

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