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Supplementary Materials for

Complex effects of inhibiting hepatic apolipoprotein B100 synthesis in humans

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The PDF file includes:

Table S7. Individual participant apoB kinetics of VLDL, IDL, LDL, and VLDL TG FCR.
References (*43–49*)

SUPPLEMENTARY MATERIALS

METHODS

Clinical protocol

Potential subjects came to the Columbia University Medical Center's Irving Institute for Clinical and Translational Research (IICTR) outpatient unit after an overnight 12 hour fast for eligibility screening. Blood and urine were obtained for determination of total cholesterol, TG, HDL-C, and LDL-C (calculated) as well as a complete blood count and chemistry tests. A complete medical history, 24-hour diet recall, ECG, and physical exam were also performed. All enrolled participants met the inclusion and exclusion criteria. The study included a ≤5-week screening period, a 1-week run-in period, a 3-week placebo phase, and a 7-9–week active treatment phase with 200 mg mipomersen. Subjects were administered placebo (9 mg sodium chloride with 0.004 mg riboflavin to match the color of the active agent, in 1 ml of water) for three weeks. At the end of the placebo phase the subjects were admitted to the IICTR inpatient unit for the first lipoprotein kinetic study.

After completion of this study, all subjects began receiving 200 mg mipomersen, in the same volume, by subcutaneous injection, once weekly for seven weeks before returning for the second lipoprotein kinetic study. After the seventh week, LDL-C levels from end of placebo and end of active treatment were measured; 15 subjects met the pre-determined criteria of ≥15 % percent reduction in LDL-C and immediately began the second kinetic study. Following a pre-specified protocol, two subjects received an additional two doses of mipomersen and, after confirmation that their LDL-C levels had decreased ≥15%, also participated in the second kinetic study. This approach was chosen to ensure adequate power with a sample size between 15 and

20 subjects who had an adequate response to mipomersen, based on intra-subject variability in FCR observed by our group and others (*38*, *43*).

There was also a 25-week post-treatment follow-up period after the final dose of mipomersen to monitor safety. The study was approved by the Columbia University Medical Center Institutional Review Board, and is registered at Clinicaltrials.gov: NCT01414881.

Lipoprotein kinetics protocol

After a 12-hour fast, subjects were admitted to the IICTR inpatient unit for 2.5 days. Fasting blood and urine were collected. A physical exam and ECG were performed. Subjects were provided with breakfast, lunch, and dinner, and then were NPO after 8 p.m. on Day 1. Starting at 1 a.m. (day 2) subjects consumed an isocaloric liquid diet (57% carbohydrate, 18% fat, 25% protein) every 2 hours throughout the kinetic study. This dietary regimen has been shown to provide stable plasma levels of lipids during the sampling period (*39*). On the morning of the second day, catheters were placed in each antecubital vein: one was used for bolus injections of [$^{13}C_6$]-phenylalanine (29.4 μmol/kg body weight), [1,1,2,3,3-$^{2}H_5$]-glycerol (100 μmol/kg body weight) (D5-glycerol) and [5,5,5-$^{2}H_3$]-leucine (D3-leucine) (9-10 μmol/kg body weight) (all isotopes from Cambridge Isotope Laboratories) followed immediately by a constant infusion of D3-leucine (9-10 μmol/kg–body weight/h) and ^{13}C-acetate (5.4 mg/kg/h) over a 15-hour period. The second catheter was used for blood sampling at 0 (pre-bolus), 20 and 40 minutes, and 1, 2, 4, 6, 8, 10, 12, 14, 15, 15.5, 16, 18, 21, and 24 hours. After the 24-hour sample, subjects were provided with breakfast and discharged. Subjects returned to our center on Day 3 for a fasting blood sample after an overnight fast. These 18 samples were used to isolate VLDL (d < 1.006 g/l), intermediate density lipoprotein (IDL) (d, 1.006-1.019 g/l), and LDL (d, 1.019-1.063 g/l) by

sequential ultracentrifugation (*31*), for measurement of plasma lipids and apolipoproteins, and for apoB, TG, and fatty acid (FA) enrichment.

Mipomersen apoB antisense

Mipomersen (KYNAMRO) is a fully phosphorothioate 20mer oligonucleotide with 5 2' methoxyethyl residues at the 5' and 3' poles and a 10 deoxynucleotide center ASO. These molecules are up to 15-fold more potent than first-generation P=S only modified drugs due to their enhanced mRNA affinity via the MOE moiety supporting an RNase H1 enzymatic termination mechanism and improved pharmacokinetic properties that permit weekly, monthly or potentially quarterly dosing. These drugs also have an improved therapeutic index due to reduced pro-inflammatory properties.

Laboratory procedures

The laboratory evaluations for hematology, blood chemistry, and urinalyses were performed by Medpace. Plasma lipid levels were measured by standard enzymatic methods on an Integra 400 Plus Analyzer (Roche Diagnostics Corp.) in the IICTR Biomarkers Laboratory. Plasma apoB was measured by an immunoturbidimetric assay on the Integra 400 Plus Analyzer and apoB in VLDL, IDL, and LDL was measured using the Assay Max Human Apolipoprotein B ELISA Kit (Assaypro) in the Biomarkers Laboratory. Plasma PCSK9 levels were measured using CircuLex Human PCSK9 Elisa Kit (CycLex Co., Ltd). Lipoprotein size and particle number were determined by ion mobility analysis (*23*).

Determination of stable isotopic enrichment of apoB and TG

The lipoprotein fractions isolated during each turnover study were used to determine stable isotopic enrichments of apoB in VLDL, IDL, and LDL; TG in VLDL; and FA in VLDL; as well as lipoprotein lipids and apolipoproteins. ApoB was isolated from VLDL, IDL, and LDL by SDS gel electrophoresis. Enrichment of each apolipoprotein with leucine and phenylalanine was measured by gas chromatography–mass spectrometry (GC-MS) (*39*). ApoB bands were excised from gels, hydrolyzed in 12-M HCl at 110°C for 24 hours, and dried under nitrogen. Two hundred fifty µl acetyl chloride/propanol solutions were added, and the samples were capped and heated at 110°C for 1 hour and dried under nitrogen gas. Samples were then incubated with hepatafluorobutyric anhydride (HFBA; Sigma-Aldrich) at 60°C for 1 hour.

Samples were reconstituted in ethyl acetate and subjected to GC-MS using a 6890 Hewlett-Packard tabletop gas chromatograph and a 5973 Hewlett-Packard mass spectrometer, equipped with negative chemical ionization capability. The enrichment of plasma free leucine and phenylalanine was assayed as well. The enrichment of TG was measured with D5-glycerol by extracting VLDL TG with Zeolite (Sigma-Aldrich) in pure isopropyl alcohol. Chloroform was added to resolubilize the TG and methanolic HCl was added to liberate glycerol and methylate the FA to methyl esters. Hexane was used to separate the glycerol and methyl esters. The liberated glycerol is isolated by liquid extraction and subsequently derivatized to tricetin (glycerol tri-acetate) by incubation with acetic anhydride. Samples are analyzed by GC-MS using positive chemical ionization with selective ion monitoring of m/z 159 and 164. The hexane layer (methyl esters) was dried and resolubilized in heptane and samples were analyzed by GC-MS using positive chemical ionization with selective ion monitoring of m/z 159 and 160.

Determination of palmitate enrichment for de novo lipogenesis measurement

DNL was determined via 1-^{13}C acetate incorporation into palmitate during steady state conditions (average of the 12- and 15-hour time points). The fraction of VLDL TG from DNL is represented by the relative enrichment of palmitate to the calculated asymptotic enrichment (100% enrichment) based on EM2/EM1 (MIDA) (*44*).

Mathematical modeling of the enrichment data

A multicompartment model that we have developed over several studies was used to estimate apoB and TG fluxes (*38, 45*). ApoB and TG were required to have the same pool structure for VLDL and the same rate constants for the VLDL pools, but with different mass distributions. The minimum number of pools needed to fit the nine sets of data (two tracers, leucine and phenylalanine, in VLDL, IDL, and LDL apoB and plasma amino acids, and one tracer, glycerol, in VLDL TG) simultaneously was chosen for the final model. The data were fitted by least squares, giving equal weight to all data points (that is, assuming a constant error variance for all measurements) using a computer program, Pool fit (*45*), which solves the differential equations in closed form and computes the fits and parameter sensitivities as sums of exponentials. The fits yielded FCRs of apoB in VLDL, IDL, and LDL, and TG in VLDL. The model also estimated rates of conversion of apoB between VLDL, IDL, and LDL. PRs were calculated by multiplying FCRs by the appropriate lipoprotein pool sizes of apoB, which were calculated as each lipoprotein's concentration of apoB in mg/ml multiplied by an estimate of plasma volume (45 ml/kg).

Markers of cholesterol synthesis and absorption

Lathosterol, campesterol, and β-sitosterol were measured by gas chromatography as previously described (*46*).

Hepatic lipase (HL) and lipoprotein lipase (LpL) measurements

Plasma was collected 15 minutes following an intravenous heparin injection (60 U/kg body weight) at the end of each treatment period following the final blood draw of the lipoprotein kinetic study for measurement of LpL and HL concentration and activity. Post heparin plasma (PHP) total lipase activities were assayed in triplicate using radiolabeled TG emulsion as a substrate (*47, 48*). Results were expressed as μmol of FA released per hour. The contribution of HL was determined by including 1-M NaCl in the assay; the activity of HL was subtracted from the total lipase activity to estimate LpL.

FA and β-hydroxybutyrate measurements

Fatty acids were quantified in serum using a colorimetric assay, and β-hydroxybutyrate was quantified in serum using a kinetic enzymatic assay, both at Medpace Reference Laboratories.

Transfection of HepG2 cells with *apoB* siRNA

Transfections were performed using Lipofectamine RNAiMax reagent following manufacturer's instruction (Invitrogen). ApoB and control siRNA were kindly provided by Merck & Co (*25*). The sequences were: *ApoB* – 5'-UUGGUAUUCAGUGUGAUGA-3'; non-targeting control – 5'-CCUGAAGAGAGUUAAAAGAU-3'. Cells were incubated for 5 hours in the transfection medium and then replaced with growth medium for 2 days before assays were applied. Quantitative real-time PCR was performed using total HepG2 cell RNA extracted with Trizol

RNA extraction reagent; cDNA was synthesized using SuperScript cDNA synthesis kits following the manufacturer's instructions (Invitrogen). Quantitative PCR (qPCR) was performed with SYBR Green in a MX 3005P (Stratagen). For steady state studies, cells were preincubated in methionine- and Cysteine-free DMEM for 1 hour and replaced with a labeling medium containing 100 μCi [^{35}S] methionine /cysteine (NEG 072014, Perkin Elmer) per each well for 2 hour. For pulse chase studies, 10 min labeling was followed by label-free incubations for given times. Newly synthesized apoB100 was isolated from cell lysates and media by immunoprecipitation with an apoB antibody and separated on 4% SDS-PAGE gel. The gels were dried down under both vacuum and heat and exposed on an X-ray film. Corresponding bands of apoB 100 were cut out from the dried gels and counted [^{35}S] in a scintillation beta counter. The effect of MTP inhibition was examined by pre-incubating cells with various concentrations of BMS-21212, provided by Bristol-Myers Squibb, prior to labeling. In studies to determine synthesis rates of apoB, cells were pre-incubated with the proteasome inhibitor, *N*-acetyl-leucyl-leucyl-norleucinal (ALLN, Sigma) at 40 μg/ml prior for one hour to labeling.

Measurements of LDL-R, IDOL, and PCSK9 expression in HepG2 cells

Total RNA was isolated using TRIzol reagent following the company protocol (15596-018, Life technologies) and used for cDNA synthesis with SuperScript II Reverse Transcription kit (15596-018, Life Technologies). Quantitative real-time PCR was done using SYBR Green PCR Master Mix and the Mx3005p Multiplex Quantitative PCR System (Agilent technologies). The relative expression of each gene was quantified by using the standard curve method and normalized by the expression of β-actin. Primers used were *LDLR* forward: 5′-GACGTGGCGTGAACATCTG-3′, reverse: 5′-CTGGCAGGCAATGCTTTGG-3′; *IDOL*

forward: 5’-GGGCTCTACCGAGCGATAAC-3’, reverse: 5’-GCCCTTCAAGTCACGGCTAT-3’; *PCSK9* forward: 5′-AGGGGAGGACATCATTGGTG-3′, reverse: 5-CAGGTTGGGGGTCAGTACC-3’.

In vivo mouse studies

Apobec-1 knockout mice were obtained from N. Davidson (Washington University) (*49*). *Apobec-1* knockout mice were housed in a pathogen-free barrier facility with a 12-hour light/dark cycle (light cycle from 7:00 to 19:00). Mice were weaned at 3 weeks of age onto chow diet (PicoLab Rodent Diet 20, LabDiet). At 12 to 16 weeks of age, some mice were changed into a HFD diet for 9 weeks. This diet was composed, by calories, of 42% fat (anhydrous milk fat), 43% carbohydrate (20% sucrose), 15% protein and, by weight, 0.15% cholesterol (TD.88137, Harland Teklad).

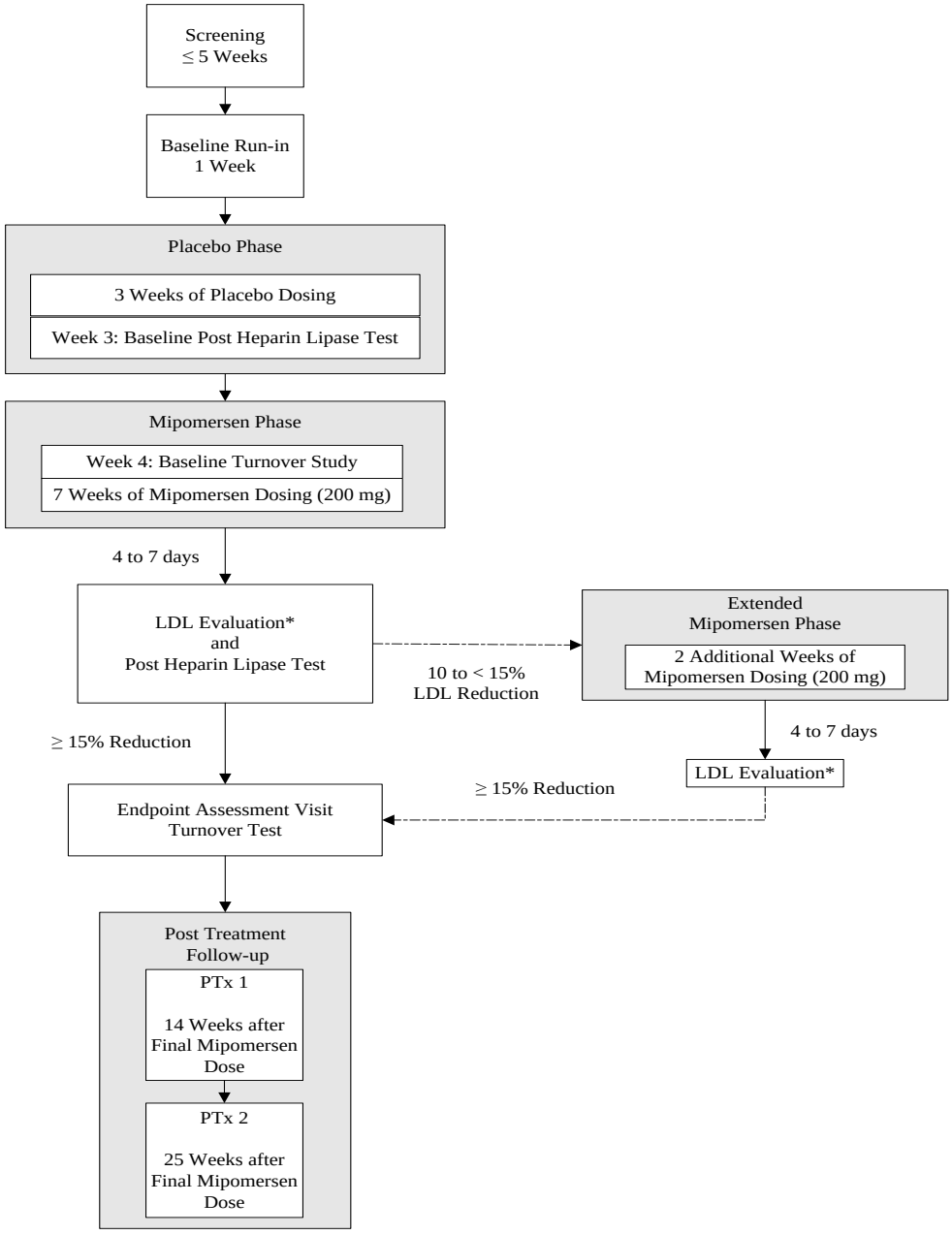
Measurements of *ApoB, Ldlr, Idol,* and *Pcsk9* gene expression and LDL-R protein in mouse liver

Liver total RNA was isolated using TRIzol reagent following the company protocol (15596-018, Life technologies) and used for cDNA synthesis with SuperScript II Reverse Transcription kit (15596-018, Life technologies). Quantitative real-time PCR was done using SYBR Green PCR Master Mix and the Mx3005p Multiplex Quantitative PCR System (Agilent Technologies). The relative expression of apoB was quantified by using the standard curve method and normalized by the expression of β-actin. Primer sequences used for qPCR were: *ApoB* forward: 5'-GCCCATTGTGGACAAGTGATC, reverse: 5'-CCAGGACTTGGAGGTCTTGGA; *β-actin* forward: 5'-GTATCCATGAAATAAGTGGTTACAGG, reverse: 5'-

GCAGTACATAATTTACACAGAAGCA. Other primers used were: *Ldlr* forward: 5′-GACGTGGCGTGAACATCTG-3′, reverse: 5′-CTGGCAGGCAATGCTTTGG-3′; *Idol* forward: 5’-GGGCTCTACCGAGCGATAAC-3’, reverse: 5’-GCCCTTCAAGTCACGGCTAT-3’; *Pcsk9* forward: 5′-AGGGGAGGACATCATTGGTG-3′, reverse: 5-CAGGTTGGGGGTCAGTACC-3’.

Western blotting for LDLR in mouse liver was performed using an antibody from Cayman Chemicals.

Supplementary Figures



*A ≥15% LDL reduction compared to Week 3 is required to proceed to the Endpoint Assessment Visit. If the subject has an LDL reduction of ≥10 to <15% after 7 weeks of mipomersen dosing, the subject can continue with 2 additional weeks of mipomersen dosing. If the subject has a <10% LDL reduction after 7 weeks of mipomersen dosing or <15% LDL reduction after 9 weeks of mipomersen dosing, the subject will be asked to return for an Early Termination Visit and post treatment follow up.

Figure S1. Study flow diagram.

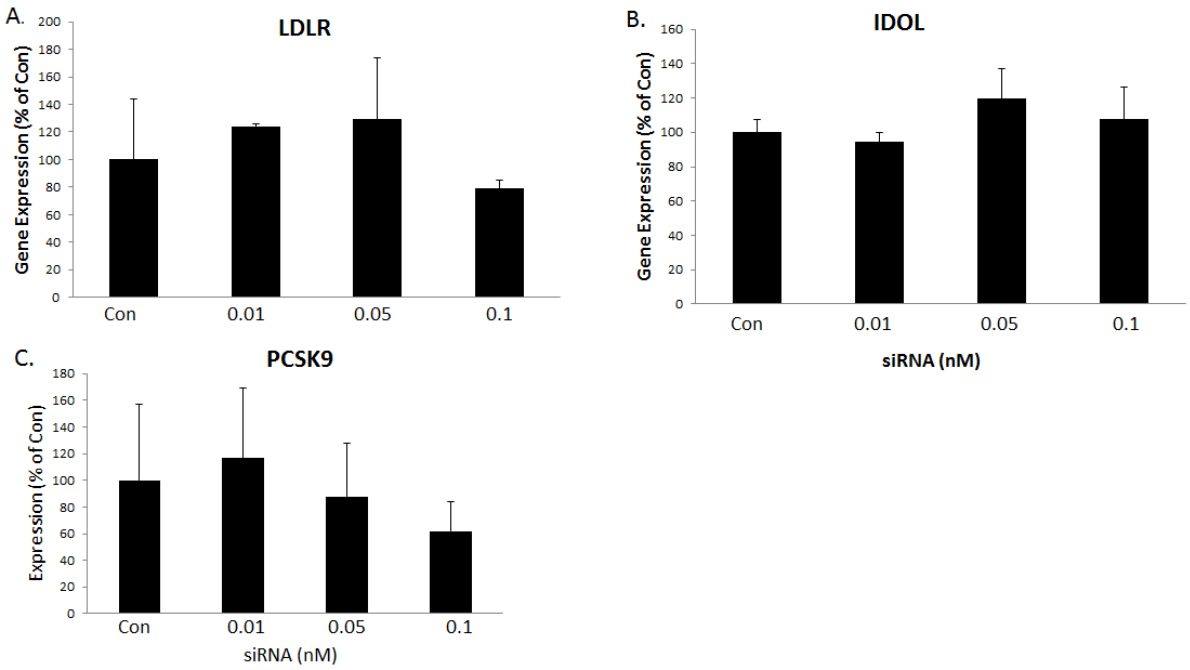


Figure S2. No changes in *LDLR*, *IDOL*, or *PCSK9* mRNA levels in HepG2 cells after knockdown of *APOB*. HepG2 cells were transfected with various concentrations of *APOB* or control siRNA for 2 days. Total RNA was extracted and gene expression levels were analyzed by qPCR. All data were normalized with *β-actin* mRNA. Experiments were performed with triplicate wells for each dose of siRNA. Data are percentages of gene expression (means ± SEM) after transfection of 0.1 nM scrambled siRNA. One-way ANOVA was used to analyze the data for differences in expression across the dose range for each gene. The absence of symbols or letters above bars indicates that the differences are not significant.

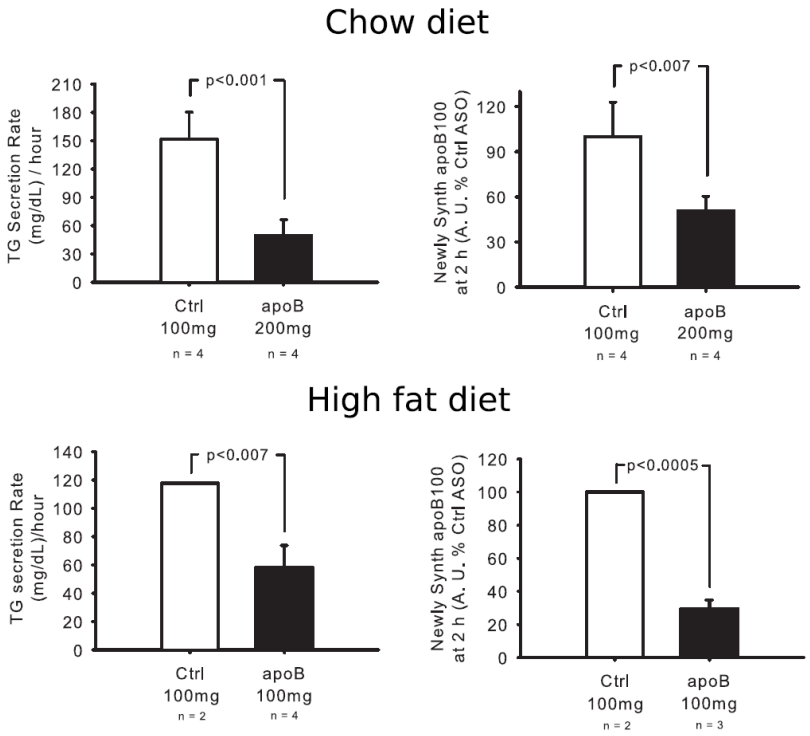


Figure S3. *ApoB* ASO inhibits hepatic secretion of TG and apoB from mice on either a chow or HFD. *Apobec-1* knockout mice were fed either a chow diet or a HFD for 9 weeks. During the last 3 weeks of feeding, mice were also injected with control (100 mg/week) or apoB ASO IP (200 mg/week for mice on chow and 100 mg/week for mice on HFD) i.p. At the end of the 3 weeks of ASO treatment, they were fasted for 4 hours and injected with Tyloxapol and [^{35}S]methionine. Blood samples were collected at 0, 30, 60, 90, and 120 minutes. TG secretion rate was determined by the change in plasma TG concentration between 30 and 120 min after Tyloxapol injection and divided by 1.5. ApoB secretion was determined from plasma ^{35}S-labeled apoB levels in counts per minute obtained at 120 min after the start of the study. For each diet group, ApoB radioactivity in mice receiving the irrelevant, control ASO was set as 100%. Data are means ± S.D. *P* values determined by independent samples t-tests.

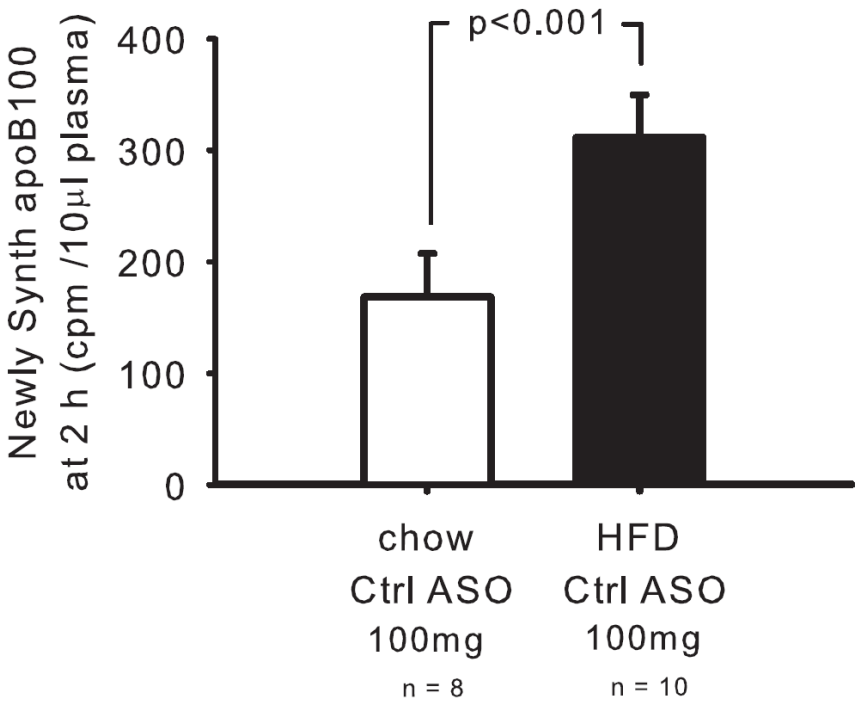


Figure S4. ApoB secretion is increased in *Apobec-1* knockout mice on HFD. Chow- and HFD-fed mice were treated for 3 weeks with control ASO. Mice were then fasted 4 hours and injected with Tyloxapol and [^{35}S]methionine intravenously. Plasma ^{35}S-labeled apoB levels in counts per minute were determined in samples obtained at 120min after the start of the study. Data are means ± S.D. *P* value determined using independent samples t-test.

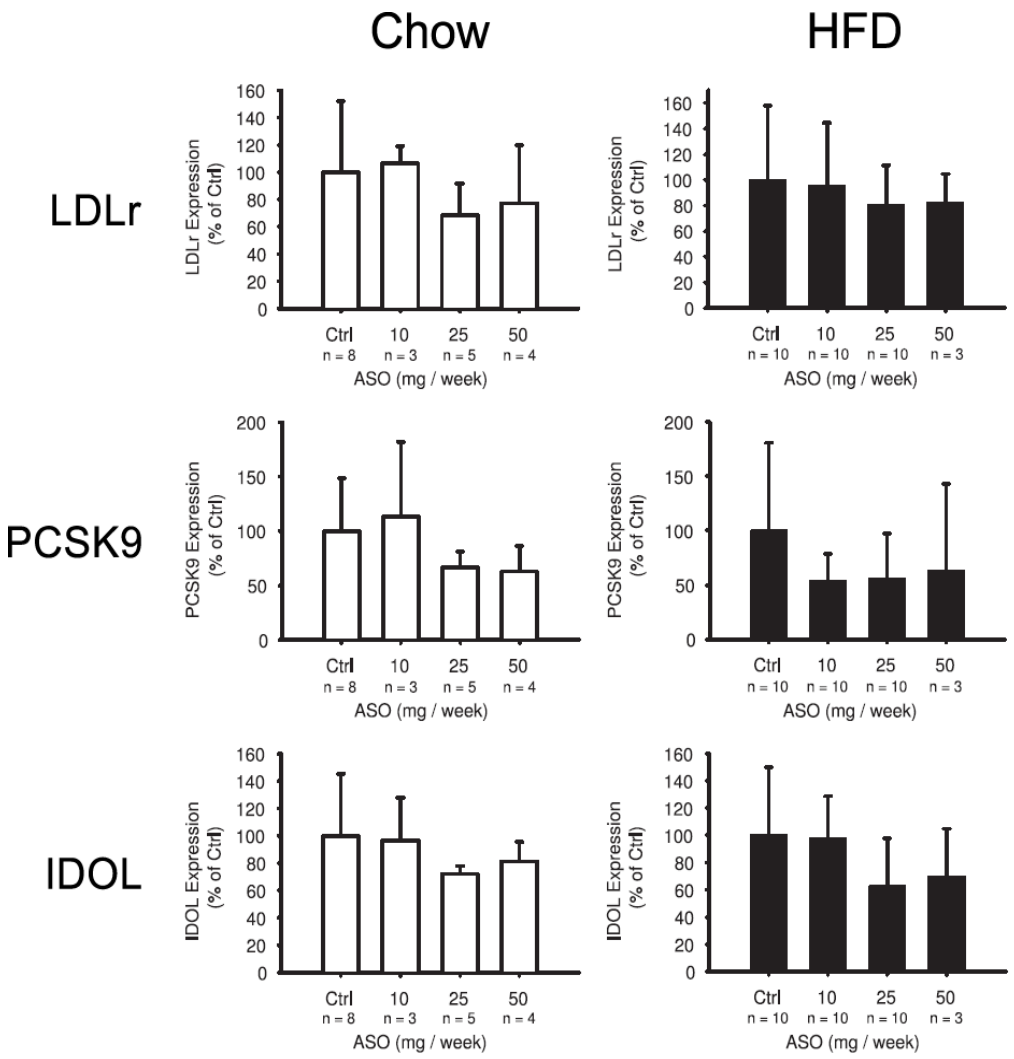


Figure S5. *ApoB* siRNA treatment does not affect expression of *Ldlr, Idol,* or *Pcsk9* in mouse livers. *Apobec-1* knockout mice on either chow or HFD for 6 weeks were injected i.p. with either control or *ApoB* ASO (25 mg/week) for 3 weeks while remaining on their diets. Gene expression in liver was determined using quantitative PCR and normalized to hepatic *β-actin* mRNA; mice were those used to measure rates of ApoB and TG secretion (Fig. 3). Data are means ± S.D. Statistical analysis was conducted using ANOVA. The absence of symbols or letters above bars indicates that the data are not significantly different.

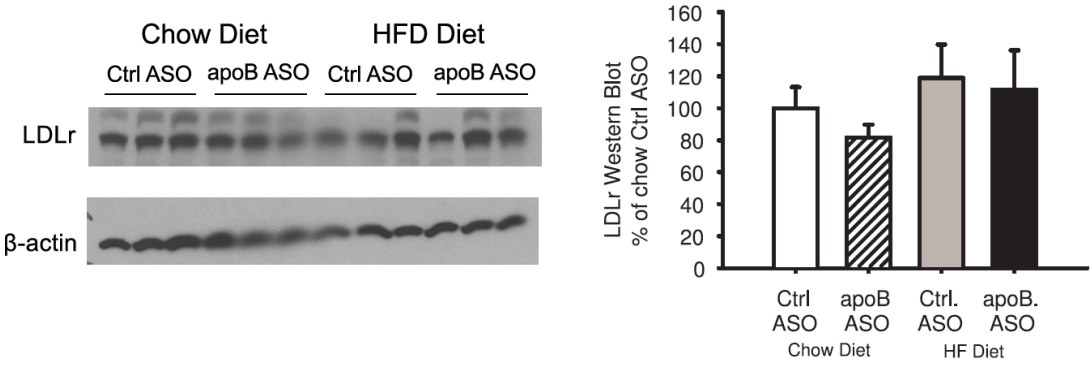


Figure S6. Hepatic LDL receptor was not affected by *ApoB* ASO. *Apobec-1* knockout mice on either chow diet or HFD for 6 weeks were injected i.p. with either control or *ApoB* ASO (25 mg/week) for 3 weeks while remaining on their diets. Livers were harvested and protein was extracted. The lower band in the Western blot for LDLR corresponds to the known molecular mass of the murine LDLR. β-Actin served as loading control. LDLR protein was quantitated by densitometry using ImageJ software (NIH) and data are means ± S.D (*n* = 3/group). Statistical analysis was performed using ANOVA. The absence of symbols or letters above bars indicates that the data are not significantly different.

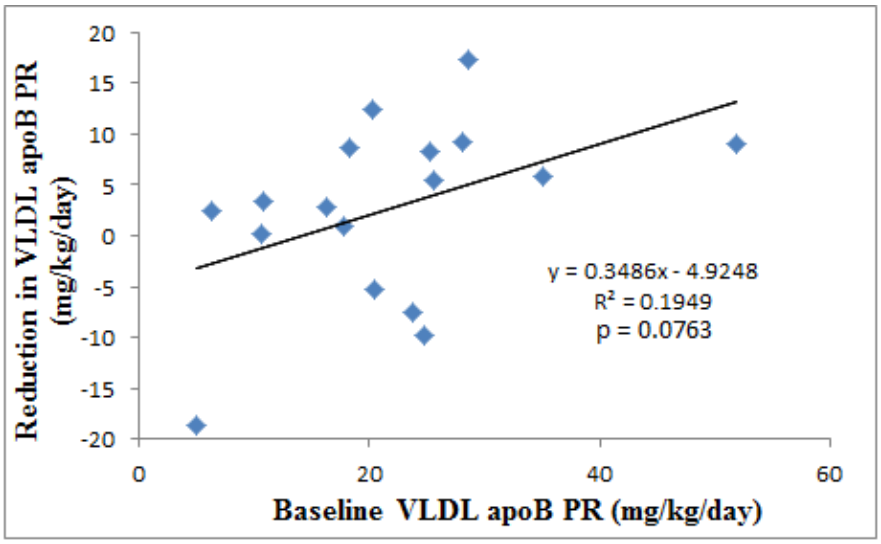


Figure S7. Relationship between mipomersen-induced reductions in VLDL apoB PR and baseline VLDL PR. The Spearman rank test was used to determine the significance of the correlation coefficient. The straight line in plot represents the best fit of the data with the equation shown on the graph.

Supplementary Tables

Table S1. Participant information. Thirty one subjects were screened; 20 enrolled; and 8 healthy men and 9 healthy women completed the study

	All screened subjects
Total screened, *n*	31
Screen failures, *n* (% of screened)	11 (35.5)
Enrolled, *n* (% of screened)	20 (64.5)
Treated with placebo, *n* (% of enrolled)	20 (100.0)
Completed placebo treatment, *n* (% of enrolled)	19 (95.0)
Discontinued placebo treatment, *n* (% of enrolled)	1 (5.0)*
Completed placebo treatment but not treated with mipomersen, *n* (% of enrolled)	1 (5.0)*
Treated with mipomersen, *n* (% of enrolled)	18 (90.0)
No additional doses (Visits 4 to 10 only), *n* (% treated)	16 (80.0)
Additional doses (Visits 11 and 12), *n* (% treated)	2 (10.0)
Completed mipomersen treatment, *n* (% of treated)	17 (85.0)
Discontinued mipomersen treatment, *n* (% treated)	1 (5.0)
Adverse event, *n* (% of treated)	1 (5.6)
Entered post-treatment follow-up, *n* (% treated)	17 (85.0)
Completed post-treatment follow-up, *n* (% treated)	17 (85.0)
Discontinued post-treatment follow-up, *n* (% treated)	0

*Physician decision.

Table S2. Effect of mipomersen on apoB lipoprotein particle number by ion mobility analysis. Data are means ± SD and percent changes are based on the difference between each parameter on placebo and mipomersen for each subject. The *P* value for percent change for each endpoint was examined by paired t-test after determining that the data were normally distributed using the Kolmogorov-Smirnov normality test.

ApoB lipoprotein	**Lipoprotein particle number (nM)**		**Change (%)**	***P*-value**
	Placebo	Mipomersen		
LDL	594.4 ± 164.9	418.9 ± 87.0	-24.7	<0.01
IDL	244.6 ± 73.4	203.3 ± 56.3	-8.0	0.12
VLDL	105.4 ± 35.4	69.4 ± 18.9	-26.9	<0.01

Table S3. Effect of mipomersen on apoB lipoprotein size distribution by ion mobility analysis. Means and percent changes are based on the difference between each parameter on placebo and mipomersen for each subject. *P* values for percent change for each endpoint were examined by paired t-test after determining that the data were normally distributed using the Kolmogorov-Smirnov normality test. The percent changes for very small VLDL were not normally distributed and, therefore, we used the Wilcoxon Rank Sum Test.

Lipoprotein	**Size range (nm)**	**Placebo distribution (%)**	**Mipomersen distribution (%)**	***P*-value**
LDL				
Large	224.6-233.30	29.5	29.0	0.77
Medium	214.1-224.6	30.0	25.6	0.01
Small	204.9-214.1	14.5	12.7	<0.01
Very small	180-205	26.0	32.7	<0.001
IDL				
IDL 1	233.30-250	40.4	36.2	<0.01
IDL 2	250-293	59.6	63.8	<0.01
VLDL				
Small	296-335	56.0	56.3	0.80
Intermediate	335-424	34.5	34.0	0.38
Large	424-547	9.6	9.8	0.83

Table S4. Effect of mipomersen on LDL size-subfraction particle numbers by ion mobility analysis. Means concentrations (± SD) and percent changes are based on the difference between each parameter, for each subject on placebo and mipomersen. *P* values for percent change for each endpoint was examined by paired t-test after determining that the data were normally distributed using the Kolmogorov-Smirnov normality test. The percent changes for LDL IIb were not normally distributed and, therefore, we used the Wilcoxon Rank Sum Test for that variable.

LDL size intervals (min-max)	**Placebo (nM)**	**Mipomersen (nM)**	**Change (%)**	***P*-value**
LDL IVc (180-190)	66.9 ± 10.5	65.8 ± 12.4	1.4	0.79
LDL IVb (190-199)	43.3 ± 6.3	38.9 ± 6.8	-8.1	0.09
LDL IVa (199-204.9)	37.6 ± 6.8	27.9 ± 4.7	-23.6	<0.001
LDL IIIb (204.9-208.20)	25.0 ± 5.8	17.1 ± 3.0	-28.2	<0.001
LDL IIIa (208.20-214.10)	60.8 ± 22.4	35.8 ± 7.3	-35.0	<0.001
LDL IIb (214.10-220.0)	84.4 ± 37.9	48.4 ± 14.1	-35.3	<0.001
LDL IIa (220-224.60)	99.0 ± 40.1	60.5 ± 21.0	-33.5	<0.001
LDLI (224.60-233.30)	177.0 ± 62.0	124.4 ± 40.5	-22.2	0.01

Table S5. Markers of cholesterol synthesis and absorption. All values were corrected for plasma cholesterol levels. *P* values for percent change for each endpoint were examined by paired t-test after determining that the data were normally distributed using the Kolmogorov-Smirnov normality test.

Plasma parameter (10^2 μmol/mmol of cholesterol)	**Placebo (mean ± SD)**	**Mipomersen (mean ± SD)**	**Change (%)**	***P*-value**
Lathosterol	136.5 ± 61.2	147.7 ± 80.1	15.9	0.27
Campesterol	174.6 ± 61.8	179.1 ± 60.0	5.6	0.40
β-Sitosterol	138.7 ± 33.3	128.6 ± 48.5	-5.0	0.54

Table S6. Hepatic lipase, lipoprotein lipase, FFA, and β-hydroxybutyrate levels. *P* values for percent change for each endpoint were examined by paired t-test after determining that the data were normally distributed using the Kolmogorov-Smirnov normality test. The percent changes for hepatic lipase and β-hydroxybutyrate were not normally distributed and, therefore, we used the Wilcoxon Rank Sum Test for those variables.

Parameter	**Placebo (mean ± SD)**	**Mipomersen (mean ± SD)**	**Change (%)**	***P*-value**
Hepatic lipase (FA/ml/h)	1.63 ± 1.13	1.02 ± 1.17	-43.5 (-83.9,0.30)	0.15
Lipoprotein lipase (FA/ml/h)	3.5 ± 1.56	2.9 ± 1.18	-2.33	0.89
Free fatty acids (mM)	0.27 ± 0.21	0.21 ± 0.11	-5.1	0.70
β-Hydroxybutyrate (mM)	0.08 ± 0.05	0.07 ± 0.02	-0.0 (0.0,0.0)	0.63

Table S7: Individual participant apoB kinetics of VLDL, IDL, LDL, and VLDL TG FCR.

Subject ID	ApoB fractional catabolic rate (FCR) (pools/day)			ApoB production rate (PR) (mg/kg/day)			TG turnover	
	VLDL	IDL	LDL	VLDL	IDL	LDL	VLDL	
							FCR (pool/day)	PR (mg/kg/h)
M01-PLACEBO	2.4	1.3	0.18	6.3	3.5	5.9	6.6	16.8
M01-MIPOMERSEN	2.8	1.2	0.33	3.8	3.3	5.3	13.4	25.7
M02-PLACEBO	6.1	5.2	0.47	28.5	12	13.4	5.4	6.4
M02-MIPOMERSEN	4.4	3.9	0.56	11.2	9.7	10.4	6.4	7.1
M04-PLACEBO	9.9	14.6	0.74	28.1	28.1	24.2	13.6	22.4
M04-MIPOMERSEN	15.5	14.1	1.2	18.9	18.9	9.1	23.4	19.9
M05-PLACEBO	6.8	5.5	0.33	10.7	5.3	8.2	5.9	10.7
M05-MIPOMERSEN	6.3	5.0	0.44	7.3	3	8.1	5.3	8.5
M06-PLACEBO	4.3	2.7	0.39	5	3.5	9.5	7.7	23.2
M06-MIPOMERSEN	16.4	6.0	0.70	23.7	11.1	12.4	7.6	12.6
M07-PLACEBO	4.3	2.5	0.53	10.6	5.7	7.2	8.0	12.8
M07-MIPOMERSEN	5.3	2.3	0.70	10.5	6.3	2.3	24.7	24.5
M09-PLACEBO	17.8	11.7	0.59	18.3	17.3	10.7	37.0	15.8
M09-MIPOMERSEN	10.1	5.3	0.75	9.6	6.3	9.4	15.2	5.3
M10-PLACEBO	9.5	9.5	0.54	20.3	20.3	8.3	5.7	3.8
M10-MIPOMERSEN	7.7	13.1	0.58	7.8	4.4	4.4	12.7	4.9
M11-PLACEBO	7.7	2.9	0.68	16.2	11.6	14	7.1	4.3
M11-MIPOMERSEN	7.8	2.1	0.81	13.4	3.9	5.9	6.9	3.5
M12-PLACEBO	4.3	5.6	0.62	23.7	18.7	13.6	6.9	10.7
M12-MIPOMERSEN	5.9	3.6	0.72	31.3	8.7	11.4	9.7	14.3
M13-PLACEBO	5.4	3.6	0.42	25.2	19.4	16	13.9	22
M13-MIPOMERSEN	7.6	5.9	0.67	17	14.9	6.5	8.7	13.2
M14-PLACEBO	12.0	4.3	0.71	35.1	13.3	10.7	12.5	10.2
M14-MIPOMERSEN	17.9	5.0	0.79	29.3	7.7	9.7	37.5	22.2
M15-PLACEBO	9.7	4.3	0.54	25.6	23.2	13.3	9.9	8
M15-MIPOMERSEN	11.9	5.3	0.50	20.2	9.6	9.1	8.8	8.3
M16-PLACEBO	8.4	6.1	0.46	24.8	23.9	13	5.3	7.4
M16-MIPOMERSEN	13.1	9.7	0.53	34.6	30	13.1	8.3	9.9
M17-PLACEBO	8.4	3.9	0.54	17.8	5.6	20.2	20.1	20.1
M17-MIPOMERSEN	7.4	4.1	0.47	16.8	8.2	9.7	17.4	15.1
M18-PLACEBO	6.2	3.7	0.51	51.8	23.2	12.5	5.4	10.4
M18-MIPOMERSEN	13.8	4.0	0.63	42.7	11.2	9.9	8.2	11.3
M19-PLACEBO	6.4	6.6	0.53	20.4	20.4	15.2	7.6	17.1
M19-MIPOMERSEN	14.4	7.6	0.75	25.6	16.8	13.5	10.8	19.8