Effects of *APOC3* Heterozygous Deficiency on Plasma Lipid and Lipoprotein Metabolism

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Objective—Apo (apolipoprotein) CIII inhibits lipoprotein lipase (LpL)-mediated lipolysis of VLDL (very-low-density lipoprotein) triglyceride (TG) and decreases hepatic uptake of VLDL remnants. The discovery that 5% of Lancaster Old Order Amish are heterozygous for the *APOC3* R19X null mutation provided the opportunity to determine the effects of a naturally occurring reduction in apo CIII levels on the metabolism of atherogenic containing lipoproteins.

Approach and Results—We conducted stable isotope studies of VLDL-TG and apoB100 in 5 individuals heterozygous for the null mutation *APOC3* R19X (CT) and their unaffected (CC) siblings. Fractional clearance rates and production rates of VLDL-TG and apoB100 in VLDL, IDL (intermediate-density lipoprotein), LDL, apo CIII, and apo CII were determined. Affected (CT) individuals had 49% reduction in plasma apo CIII levels compared with CCs ($P<0.01$) and reduced plasma levels of TG (35%, $P<0.02$), VLDL-TG (45%, $P<0.02$), and VLDL-apoB100 (36%, $P<0.05$). These changes were because of higher fractional clearance rates of VLDL-TG and VLDL-apoB100 with no differences in production rates. CTs had higher rates of the conversion of VLDL remnants to LDL compared with CCs. In contrast, rates of direct removal of VLDL remnants did not differ between the groups. As a result, the flux of apoB100 from VLDL to LDL was not reduced, and the plasma levels of LDL-cholesterol and LDL-apoB100 were not lower in the CT group. Apo CIII production rate was lower in CTs compared with CCs, whereas apo CII production rate was not different between the 2 groups. The fractional clearance rates of both apo CIII and apo CII were higher in CTs than CCs.

Conclusions—These studies demonstrate that 50% reductions in plasma apo CIII, in otherwise healthy subjects, results in a significantly higher rate of conversion of VLDL to LDL, with little effect on direct hepatic uptake of VLDL. When put in the context of studies demonstrating significant protection from cardiovascular events in individuals with loss of function variants in the *APOC3* gene, our results provide strong evidence that therapies which increase the efficiency of conversion of VLDL to LDL, thereby reducing remnant concentrations, should reduce the risk of cardiovascular disease.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2019;39:63-72. DOI: 10.1161/ATVBAHA.118.311476.)**

Key Words: apolipoprotein C-III ■ cardiovascular diseases ■ isotopes ■ lipolysis ■ lipoprotein lipase

Apo (apolipoprotein) CIII was first isolated and characterized nearly 50 years ago by Brown et al.[1] Soon after, it was shown that apo CIII was an inhibitor of LpL (lipoprotein lipase),[2] an action opposing the activity of another apoprotein; apo CII, the necessary activator of LpL.[3] The role of apo CIII in lipoprotein metabolism was later expanded by studies in perfused rat livers demonstrating that apo CIII inhibits uptake of triglyceride (TG) rich lipoproteins and remnants.[4,5] The identification of 2 sisters with complete absence of apo CIII because of a homozygous chromosomal deletion that also included apo AI, apo AIV, and apo AV,[6] allowed us to demonstrate, in vivo, that absence of apo CIII resulted in a dramatic increase in lipolysis of VLDL (very-low-density lipoprotein)-TG.[7] Studies in mice overexpressing apo CIII or with targeted deletions of the *APOC3* gene confirmed the human findings and also supported the role of apo CIII as an inhibitor of hepatic uptake of apoB100-containing lipoproteins.[8–11] In addition, mouse- and hepatoma-based studies suggested that apo CIII may increase the incorporation of TG into nascent VLDL particles.[10,12]

Although interest in apo CIII's role in lipid metabolism has continued, enthusiasm for apo CIII as a therapeutic target was limited by uncertainty about the relationship between hypertriglyceridemia and risk for cardiovascular disease (CVD).[13,14] Recent genetic studies using both genome-wide association and Mendelian randomization approaches have, however, established the relationship between loss of function of apo CIII,

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Nonstandard Abbreviations and Acronyms	
Apo	apolipoprotein
CVD	cardiovascular disease
FCR	fractional clearance rates
HDL	high-density lipoprotein
IDL	intermediate-density lipoprotein
LDL	low-density lipoprotein
LpL	lipoprotein lipase
PR	production rates
TG	triglyceride
VLDL	very-low-density lipoprotein

which results in lower plasma TG concentration, and reduced CVD risk.[15,16] We found that 5% of the Lancaster Old Order Amish are heterozygous carriers of a null mutation, R19X (HGVS NM 000040.2 c.55C>T p.Arg19Ter rs76353204) in the *APOC3* gene, which converts an arginine to a termination codon, resulting in a 50% reduction of plasma apo CIII levels.[17] In addition to having lower fasting and postprandial TG, higher levels of HDL (high-density lipoprotein)-cholesterol, and lower levels of LDL (low-density lipoprotein)-cholesterol, heterozygous deficient individuals have less subclinical atherosclerosis, as determined by coronary artery calcification.[17] Our report was followed by large cohort studies of *APOC3* loss of function mutations, including R19X, which demonstrated a 40% reduction in myocardial infarction in carriers.[18,19]

Based on the nonhuman mechanistic studies described above, heterozygous loss of function of *APOC3* might reduce risk for CVD by any or all of the following: (1) reducing secretion of VLDL from the liver, (2) increasing hepatic uptake of VLDL and chylomicron remnants by the liver, and (3) increasing lipolytic conversion of VLDL to LDL. We used stable isotope tracers to determine which of these 3 possible results of heterozygous loss of function of *APOC3* would be most important in determining the differences in plasma lipids between R19X affected Amish and their unaffected siblings. Our findings support the development of therapies that lower plasma apo CIII levels as a means of treating moderate to severe hypertriglyceridemia[20,21] which may reduce risk for CVD.

Materials Methods

The data that support the findings of this study are available within the article and its online-only Data Supplement.

Study Subjects

We recruited 5 participants heterozygous for the *APOC3* R19X mutation, hereafter denoted by CT, and 5 sex-matched, unaffected siblings, hereafter denoted by CC, ages 35 to 71 years, from the Lancaster Old Order Amish population.[17] Participants were not receiving lipid-altering medications. All study participants provided written informed consent and the studies were approved by the Institutional Review Boards of the University of Maryland School of Medicine and Columbia University Medical Center.

Stable Isotope Kinetic Studies

The 2 sibs, in each pair, were studied on the same day at the Amish Research Clinic in Lancaster, PA. The protocol for these studies was one that we have used previously,[22] with minor modifications to allow subjects to complete some visits in their homes. On day 1, participants fasted for 12 hours, after which a nurse visited their homes and drew baseline bloods for safety and fasting lipid and lipoprotein measurements. After 6 PM on day 1, they were NPO (nothing per mouth [oral]) until 11 PM when they started a liquid, isocaloric, 18% fat diet that was provided every 2 hours for the next 30 hours. At 5:30 AM (day 2), they arrived at the Amish Research Clinic, where 2 intravenous were placed in antecubital veins of each arm and baseline bloods were drawn (time 0 hour). Immediately after, boluses of $^{2}H_3$-L-leucine (10 µmol/kg BW), Ring-$^{13}C_6$-L-phenylalanine (29.4 µmol/kg BW), and $2H_5$-glycerol (100 µmol/kg BW) were administered over a 10-minute period, followed by a constant infusion of $^{2}H_3$-L-leucine (10 µmol/kg BW per hour) over 15 hours. Additional blood samples were collected at 20 and 40 minutes, and at 1, 2, 4, 6, 8, 10, 12, 14, 15, 15.2, 15.4, 16 hours after the administration of tracers and processed to isolate plasma and serum. After the 16 hours blood sample, the subjects returned to their homes where they continued to consume the liquid meal protocol. Eight hours later a nurse drew the final 24 hours blood sample in their homes. VLDL, IDL (intermediate-density lipoprotein), LDL, and HDL were obtained from the 16 plasma samples via sequential density ultracentrifugation.

Determination of Stable Isotope Enrichment of ApoB100 and TG

The isolated lipoprotein fractions were used to determine stable isotopic enrichments of $^{2}H_3$-L-leucine and Ring-$^{13}C_6$-L-phenylalanine in apoB100 in VLDL, IDL, and LDL. ApoB100 was isolated from VLDL, IDL, and LDL by SDS-polyacrylamide gel electrophoresis. The isolated apoB100 bands were excised from the gels, hydrolyzed, and the amino acids derivatized. Plasma free amino acids were recovered from 0.25 mL plasma after precipitation of proteins with acetone and extraction of the aqueous phase with hexane. The aqueous phase was dried under vacuum, amino acids were derivatized. Enrichments of [5,5,5-$^{2}H_3$]-leucine and [$^{13}C_6$]-phenylalanine tracers in apoB100-lipoproteins and plasma free leucine and phenylalanine were measured by gas chromatography-mass spectrometer using an Agilent 6890 gas chromatography and a 5973 mass spectrometer with negative chemical ionization. Additionally, kinetic analysis of TG in VLDL was performed with $2H_5$-glycerol. TG was separated from phospholipid by zeolite binding. The TG was resolubilize with chloroform. We performed transesterfication with methanolic HCL. Glycerol was isolated by liquid/liquid extraction (hexane and water added) and derivatized to triacetin (glycerol triacetate) through incubation with acetic anhydride. We performed gas chromatography-mass spectrometer positive chemical ionization with selective ion monitoring of m/z 159 and 164.

Compartmental Modeling of ApoB100 and TG Metabolism

Fractional clearance rates (FCRs) and production rates (PR) of TG and apoB100 in VLDL, and of apoB100 in IDL and LDL were determined using a compartmental model to fit stable isotope enrichment data.[23–25] In our general model, apoB100 and TG are required to have the same pool structure and the same rate constants for each VLDL pool, but with different mass distributions. With >1 pool in the VLDL fraction, the different mass distributions lead to different VLDL-FCRs for TG and apoB, since VLDL-FCR is obtained as a weighted average of the individual FCRs (the weights given by the mass distribution). However, if there is only one pool in the VLDL fraction, TG and apoB necessarily have the same FCR. For each study, the minimum number of pools needed to simultaneously fit the 9 sets of data (2 tracers, leucine and phenylalanine, in VLDL-, IDL-, and LDL-apoB100 and plasma amino acids, and 1 tracer, glycerol, in VLDL-TG) is chosen for the final model. In the present study, the final model had 1 pool each for VLDL, IDL, and LDL. The data were fitted by least squares, giving equal weight to all data points (ie, assuming a constant error variance for all measurements) using a computer program, Pool fit,[23] which solves the differential equations

in closed form and computes the fits and parameter sensitivities as sums of exponentials. The fits yielded fractional clearance rates (FCRs) of apoB100 in VLDL, IDL, and LDL, and TG in VLDL. The model also estimated rates of conversion of apoB100 between VLDL, IDL, and LDL. PRs (mg/kg per day) were calculated by multiplying FCRs (in pools/day) by the appropriate lipoprotein pool sizes of apoB100, which were calculated as each lipoprotein's concentration of apoB100 in mg/dL multiplied by an estimate of each individual's plasma volume (45 mL/kg). Schematics of the models used to analyze apoB100, TG-glycerol, and apo CIII in the present study are included in the supplement (Figure I in the online-only Data Supplement). Based on the best fit of the present data, we have only one VLDL pool for apoB and TG, direct conversion of VLDL-apoB to LDL, as well as conversion from VLDL to LDL via IDL, no direct out pathway from IDL, and a small component of direct secretion of LDL from the liver.

Determination of Stable Isotope Enrichment of Apo CII and Apo CIII

Apo CIII enrichment with $2H_3$-L-leucine was measured using Ultraperformance liquid chromatography-mass spectrometry. VLDL and HDL fractions were digested with trypsin as previously described.[26] A multiple reaction monitoring method was used to determine the following precursor-product ion transitions for a peptide specific to apo CIII (GWVTDGFSSLK; M0: 599.0>854.5; M3: 600.5>857.6) and apo CII (TYLPAVDEK; M0: 518.4>658.4; M3: 519.9>658.4).

Compartmental Modeling of Apo CII and Apo CIII Metabolism

The $^2H_3$-leucine enrichment data for apo CII and apo CIII in HDL or VLDL was fitted by a single pool with the VLDL-apoB100 enrichment plateau used as the best available estimate of the liver leucine pool enrichment. Separate FCRs were estimated for HDL and VLDL. While the enrichment of apo CIII was different between HDL and VLDL, there was a constant ratio across time-points in any single study. This means that the FCR of apo CIII is estimated to be the same in VLDL and HDL; any difference is solely because of random measurement error. We ascribe this to apo CIII moving freely among lipoproteins.[27] The situation was similar with apo CII. Therefore, we a calculated single plasma FCR for apo CIII and apo CII. Apo CIII and apo CII PRs were calculated using the FCR for each apolipoprotein and its plasma pool size, as described above for apoB.

Quantitation of Apo CIII in VLDL and HDL Fractions

Apo CIII was quantitated by Ultraperformance liquid chromatography-mass spectrometry, using a Waters Xevo TQS triple quad mass spectrometer coupled with an Acquity UPLC (Waters, Milford, MA). VLDL and HDL fractions were digested with trypsin as described previously.[28] In brief, 200 µL of ultracentrifuged VLDL or HDL from each time-point was desalted, reduced with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin overnight. A multiple reaction monitoring method was used to determine the following precursor-product ion transitions for a peptide specific to apo CIII (GWVTDGFSSLK; M0: 599.0>854.5) and a deuterated internal standard (M8: 603.0>862.5). Lower limit of quantitation, defined as the level at which the residual of the calibration line is <20% of the expected concentration was determined to be 1 nm. The intra-assay precision for the assay was 4.00%. Apo CII was similarly quantitated using the following precursor-product ion transitions for a peptide specific to apo CII (TYLPAVDEK; M0: 518.4>658.4; M3: 519.9>658.4). The proportions of plasma apo CIII and apo CII residing in VLDL and HDL were then calculated.

Biochemical and Immunologic Assays

Day 1 blood was collected after a 12-hour overnight fasting period. Additional timed blood samples were collected while the subjects were consuming the liquid diet, both before (0 hour) and at various time-points after the stable isotope infusion was started (20 and 40 minutes, 1, 2, 4, 6, 8, 10, 12, 14, 15, 15.2, 15.4, 16, and 24 hours.). Plasma cholesterol, TG, and HDL cholesterol were measured by Integra400plus (Roche). Plasma LDL-cholesterol levels were estimated using the Friedewald formula. Cholesterol and TG were also measured enzymatically in VLDL, IDL, LDL, and HDL isolated by ultracentrifugation. Plasma apo CII, apo CIII, and apoE were measured by human ELISA kits (ab168549 [apo CII]; ab154131 [apo CIII]; ab108813 [apoE], Abcam, Cambridge, MA). ApoB100 in plasma and in VLDL, IDL, and LDL was measured using an apoB100 ELISA kit (A70102 AlerCheck, Inc).

Statistical Analysis

The data are presented as means and SD. The mutation effects were assessed by analyzing within-pair differences for statistical significance using paired *t* tests. The primary end point was the percent difference in FCR of VLDL-TG and VLDL-apoB100 between affected and unaffected sib-pairs, and P=0.05 was considered significant. A key secondary end point was the partition of VLDL between conversion to LDL and hepatic uptake, and P=0.01 was considered significant. All other comparisons were exploratory.

Results

Study Population

We enrolled 10 participants (5 CT and 5 CC), including 3 affected males, 2 affected females, and sex- and age-(within 10 years) matched unaffected siblings. Mean ages (CT 50±11.1, CC 52.4±12.4) and body mass index (CT 28.6±4.3, CC 26.9±5.9) were similar between the groups (Table1).

Plasma Lipid and Apolipoprotein Levels

Plasma TG levels were lower in the CT subjects (61±29 mg/dL) versus their CC siblings (92±26 mg/dL; Table 1). The mean (±SD) percent difference for TG between the pairs was 35±20 (P<0.02). Total plasma cholesterol levels did not differ between sib-pairs. Although we have previously shown that *APOC3* R19X carriers have higher HDL and lower LDL-cholesterol levels,[17] in this much smaller sample, the levels of cholesterol in these lipoproteins were not significantly different between the pairs. Plasma levels of apoB100 and apoE were not different between the 2 groups, but, as expected, apo CIII levels were significantly reduced in the CT group (CT 61±18 µg/mL versus CC 123±33 µg/mL) with a mean percent difference between pairs of 49±19 (P<0.01). Of interest, apo CII levels were also lower in the affected (48±23 µg/mL) compared with the unaffected siblings (71±25 µg/mL), with a mean percent difference of 35±14 (P<0.01). The percent of plasma apo CIII in VLDL was similar in the CT (23±9%) and CC (28±9%) groups. The percent of plasma apo CII in VLDL was also similar in the CT (30±14%) and CC (35±10%) groups.

The cholesterol and TG levels in the isolated VLDL fractions (Table 2) were lower in the CT (4±2.0 mg/dL and 18±12 mg/dL, respectively) than in their CC siblings(7±3 mg/dL and 32±11 mg/dL, respectively; P<0.03 for cholesterol and P<0.02 for TG). The cholesterol and TG levels in the IDL

Table 1. Baseline Characteristics and Plasma Lipid and Apolipoprotein Levels of *APOC3* R19X Carriers and Unaffected Sib-Pairs

ID No.	Sex	Age, y	BMI	Chol, mg/dL	TG, mg/dL	LDL-C, mg/dL	HDL-C, mg/dL	ApoB100, mg/dL	Apo CIII, μg/mL	Apo CII, μg/mL	ApoE, μg/mL
CC1	F	55	34	181	62	92	78	105	140	45	52
CC2	F	71	18	167	120	100	44	70	138	94	223
CC3	M	53	26	303	117	219	60	142	85	101	172
CC4	M	37	30	179	88	98	63	66.2	160	63	236
CC5	M	48	27	137	73	84	39	66	92	53	94
Mean±SD		52±12	27±6	193±64	92±26	119±56	57±16	90±34	123±33	71±25	155±81
CT1	F	46	27	145	25	76	63	53	30	22	165
CT2	F	63	32	215	103	127	68	87	75	73	160
CT3	M	50	34	220	67	138	69	120	69	70	154
CT4	M	35	28	160	61	92	57	64	63	45	164
CT5	M	57	23	173	48	93	70	105	66	28	92
Mean±SD		50±11	29±4	183±33	61±29	105±26	65±5	86±28	61±18	48±23	147±31
%Difference (SD)		−2.3 (22.1)	13.7 (40.9)	−1.6 (20.8)	−35.2 (20.1)*	−4.9 (±22.9)	22.3 (34)	−2.6 (17.2)	−49.1 (18.5)†	−35.1 (13.8)†	17.0 (55.5)

Participants are defined as CC (unaffected siblings) and CT (affected siblings), in order of sib-pairs. All lipid and lipoprotein levels were obtained from 5 time-points during the 24-h period of the stable isotope studies. Data are presented as means and SD of absolute concentrations as well as means and SD of the percent differences between the 2 groups. Statistical significance of the percent differences was assessed using paired *t* tests. Apo indicates plasma apolipoprotein; BMI, body mass index; Chol, plasma cholesterol; TG, plasma triglycerides; LDL-C, plasma low-density lipoprotein cholesterol; HDL-C, plasma high-density lipoprotein cholesterol.

*Significance at $P<0.02$.

†Significant at $P<0.01$.

and LDL fractions were not different between the sib-pairs. Despite similar plasma apoB100 levels, VLDL-apoB100 levels were lower in the CT (2±0.5 mg/dL) compared with the CC (4±2 mg/dL), with a mean percent difference between pairs of 36±48 ($P<0.05$). There were no differences in IDL- or LDL-apoB100 levels between the sib-pairs.

ApoB100 and TG Metabolism

As noted in Methods, we modeled VLDL-TG and VLDL-apoB100 metabolism jointly, and the best fits of the stable isotope enrichment data required only a single VLDL pool. Thus, the VLDL-apoB100 FCR and VLDL-TG FCR in each subject were the same. The results demonstrate that the lower levels of VLDL-TG and VLDL-apoB100 in the CT group were because of a significantly greater FCR of VLDL-TG and VLDL-apoB100 (% increase 116±31, $P<0.001$; Table 3). The VLDL-apoB100 PRs were similar in the CT and CC siblings (27±7 and 26±12 mg/kg per day, respectively), as were the VLDL-TG PRs (790±291 and 555±156 mg/kg per day). The ratio of VLDL-TG PR to VLDL-apoB PR, an indicator of the size of newly secreted VLDL particles, did not differ between the groups (CT 32±13 versus CC 27±19).

We also found that the FCR of IDL-apoB100 was greater in the CT individuals compared with their CC siblings (% increase 203±223; $P<0.02$). We did not observe differences between sib-pairs in the FCR of LDL-apoB100 or in the PRs of IDL- or LDL-apoB100. There were 3 sources of LDL-apoB: conversion of VLDL to LDL via IDL, direct conversion of VLDL to LDL, and direct secretion of LDL from the liver. These made up, respectively on the average, 31%, 62%, and 7% of LDL-PR in the CT siblings and 33%, 55%, and 12% in the CC siblings; none of the sources differed significantly between the groups (Table 3).

A key secondary goal of the study was to determine the effects of reduced apo CIII levels on the partitioning of VLDL-apoB100 flux between conversion of VLDL to LDL and direct

Table 2. Effects of *APOC3* R19X on Mean Levels of Lipoprotein Lipids and ApoB100 Concentrations

	CC (mean±SD), mg/dL	CT (mean±SD), mg/dL	Percent Differences, (mean±SD)
VLDL-Chol	6.9±3.4	3.5±1.9	−47.9±13.3*
IDL-Chol	3.07±2.9	1.47±1.0	−37.3±35.8
LDL-Chol	61.7±24.0	56.6±13.6	−4.1±22.7
VLDL-TG	31.6±11.0	17.7±11.7	−45.3±26.6*
IDL-TG	3.12±2.0	1.89±1.1	−36.1±24.5
LDL-TG	9.6±3.8	8.6±2.2	−6.1±26.0
VLDL-apoB100	4.29±2.1	2.0±0.47	−36.4±47.6*
IDL-apoB100	1.96±1.9	1.07±0.66	−24.6±41.7
LDL-apoB100	51.2±15.3	58.0±22.6	−13.6±32.9

Participants are defined as CC (unaffected siblings) and CT (affected siblings). Data were obtained from 5 time-points during the 24-h period of the stable isotope studies. Data are presented as means and SD of absolute concentrations as well as the means and SDs of the percent differences between CC and CT groups. Statistical significance of the percent differences was assessed using paired *t* tests. ApoB100, apolipoprotein B100; chol, cholesterol; TG, triglycerides; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; and LDL, low-density lipoprotein.

*Significant at $P<0.05$.

Table 3. Effects of *APOC3* R19X Mutation on Kinetic Parameters for TG and ApoB100 Metabolism

	CC±SD	CT±SD	% Difference±SD
VLDL-TG and VLDL-apoB100 FCR, pools/d	15.0±6.0	31.0±8.9	116±31*
IDL-apoB100 FCR, pools/d	5.9±3.1	12.2±2.6	203±223†
LDL-apoB100 FCR, pools/d	0.63±0.31	0.77±0.18	37±43
VLDL-TG PR, mg/kg per d	555±156	790±291	45.9±56.9
VLDL-apoB100 PR, mg/kg per d	26.0±12.4	26.8±6.7	34±93
IDL-apoB100 PR, mg/kg per d	4.0±3.1	5.6±3.5	122±222
LDL-apoB100 PR, mg/kg per day	14.8±8.7	19.1±5.6	58±72
LDL-apoB PR from VLDL via IDL, mg/kg per d	4.0±3.1	5.6±3.5	
LDL-apoB PR directly from VLDL, mg/kg per d	8.4±9.8	11.9±4.8	
LDL-apoB PR from the liver, mg/kg per d	2.4±5.3	1.5±2.3	
VLDL-TG PR/VLDL-apoB100 PR	27.3±19.1	31.6±13.2	37.7±71.7

Participants are defined as CC (unaffected siblings) and CT (affected siblings). Data derived from compartmental modeling of stable isotope enrichment of samples obtained over a 24 h. period. Data are presented as means and SD of the absolute FCRs and PRs as well as the means and SD of the percent difference in each parameter between the CT and CC groups. Statistical significance of the percent differences was assessed using paired *t* tests. ApoB indicates apolipoprotein B100; FCR, fractional clearance rate; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; PR: production rate; TG, triglycerides; and VLDL, very low-density lipoprotein.

†Significant at $P<0.02$.

*$P<0.001$.

removal of VLDL by the liver. The Figure depicts a model of apoB100-lipoprotein transport from the liver to circulating VLDL, and then to either LDL or back to the liver. Inherent to this model is the concept that the initial LpL-mediated lipolysis of VLDL-TG generates a smaller particle with less TG than present at the time of secretion into the circulation, and at some point in this process, the particle has lost enough TG that it has a density >1.006 and, therefore, leaves the VLDL pool. This particle can be found in the IDL and LDL pools or be irreversibly taken up by the liver. It is important to understand that although we show a single VLDL pool in the Figure, it comprises a range of VLDL of differing size and number of TG molecules: the largest and most TG-rich VLDL are those that have just entered the circulating VLDL pool and the smallest and most TG-poor are those that have undergone lipolysis and are about to either leave the circulation directly or become IDL or LDL. We show a single pool, despite the heterogeneous nature of the population of VLDL within this pool, because when we used a model with >1 VLDL pool, it did not improve the fit of our kinetic data. As noted above, the rates of secretion of newly synthesized VLDL from the liver (equal to VLDL-PR) were similar for CT and CC siblings and, therefore, the blue arrows from the liver to VLDL in each group are the same. However, because lipolysis of VLDL-TG was much faster in the CTs (depicted by the much thicker black arrow coming out from VLDL in that group), the size of the VLDL pool in CTs was about 36% smaller than the VLDL pool of CCs (Table 2). The green and red arrows represent the number of VLDL plasma pools each day that, after lipolysis of TG, move either to LDL or to the liver, respectively. Our compartmental analysis indicated that there was a significant 12.3±6.3 pools/day difference in the FCRs between CT and CC sib-pairs for the conversion of VLDL-apoB100 to IDL and LDL ($P=0.01$), but only a 3.6±7.3 pools/d difference in FCRs between the 2 groups for direct hepatic removal of those particles ($P=0.33$). Thus, only the conversion rate of VLDL to LDL, for example, the lipolytic pathway, was significantly greater in the group with partial loss of apo CIII. This is depicted by the thicker green arrow in CT compared with CC, whereas the red arrows are similar in the 2 groups (Figure). The individual and mean data for these parameters are presented in Table 4. This greater rate of conversion of VLDL to IDL and LDL versus uptake by the liver was reflected by a nonsignificant increase in LDL-apoB100 PR in the CT compared with the CC group (Table 3).

As noted above, the generation of LDL from VLDL occurred via direct conversion of VLDL to LDL or conversion of VLDL to IDL followed by conversion of the latter to LDL, with direct conversion accounting for the largest proportion of LDL generated. We were able, using our model, to determine the FCRs of each of these pathways in the CT and CC groups, and these data are presented in Table 4. The FCR for direct conversion of VLDL to LDL was significantly greater (14.8±9.1 pools/d) in the CT siblings than in the CC group (5.5±5.7 pools/d; $P=0.04$). There was no difference in the FCRs for conversion of VLDL to LDL via IDL between the 2 groups (CT: 6.5±4.0; CC: 3.5±4.6 pools/d; $P=0.2$). These results, together with those for the FCRs of overall conversion to LDL versus direct uptake of VLDL, support much greater lipolytic activity in the affected versus the nonaffected sib-pairs.

Apo CIII and Apo CII Metabolism

Previous studies by several groups have demonstrated that the kinetics of rapidly exchangeable apolipoproteins such as apo CIII and apo CII[29] can only be characterized by plasma FCRs and PRs.[30–32] However, because of a lack of unanimity on this issue,[33] we determined enrichments of each apolipoprotein in both VLDL and HDL, which together transport nearly all of these 2 proteins in plasma. We found essentially identical FCRs for both apo CIII and apo CII in VLDL and HDL (Table I in the online-only Data Supplement) and, therefore, have presented only a single FCR and PR for each apolipoprotein in Table 5. In the CC group, the FCRs of apo CIII (1.3±0.7 pools/d) and apo CII (1.4±0.8 pools/d) were similar, consistent with a common clearance pathway of each of these apolipoproteins.[34] On the other hand, the PR of apo CIII (7.4±6 mg/kg per day) was almost double that of apo CII (3.9±2 mg/kg per day), indicative of unique regulation of the synthesis of these 2 apolipoproteins. Similar differences in the PRs of apo CIII and apo CII have been reported previously.[34] In the CT group, the FCRs of

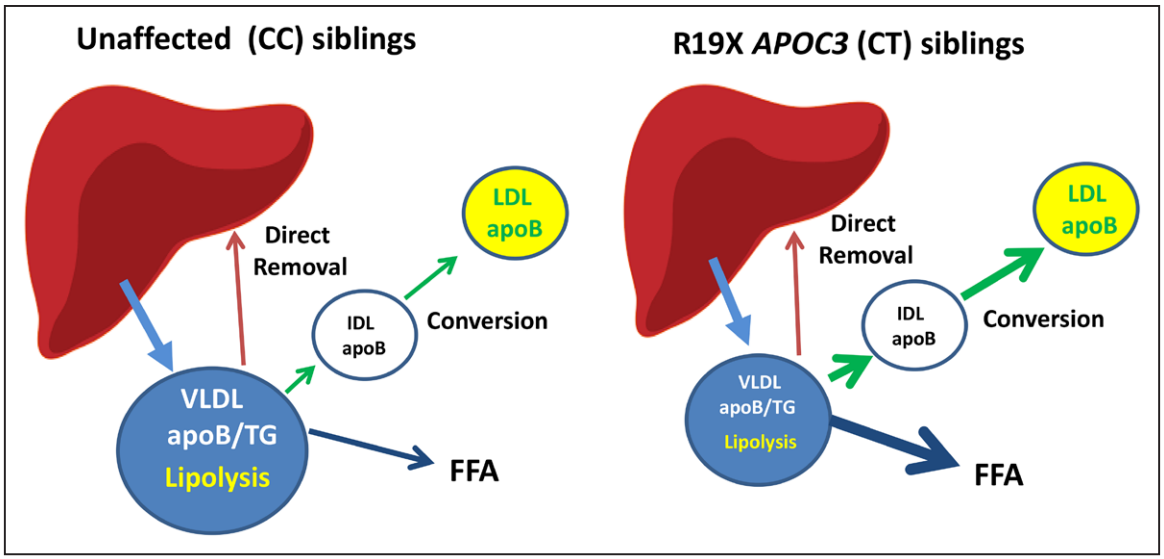

Figure. The figures depict the effects of partial loss of apo CIII (apolipoprotein CIII) synthesis of the metabolism of VLDL (very-low-density lipoprotein). Although the rates of secretion of VLDL-apoB100 and VLDL triglyceride (TG; blue arrows) are similar in CC (unaffected siblings) and CT (affected siblings), the size of the VLDL plasma pool (blue circles) is reduced by 36% in CT because an increase in LpL (lipoprotein lipase) mediated lipolysis of VLDL-TG (black arrows) leads to a doubling of the fraction clearance rate of apoB100 and TG from the VLDL pool. The lipolysis of VLDL-TG generates particles that either undergo conversion to IDL (intermediate-density lipoprotein) and LDL (green arrows) after additional lipolysis of TG by LpL or hepatic lipase or direct removal by the liver (red arrows). The rate of conversion of VLDL to LDL was significantly greater in CT compared with CC whereas the rates of direct removal were similar in the 2 groups (Table 4). These data indicate that, in individuals heterozygous for loss of function of APOC3, who have normal levels of lipoprotein lipase, reduced levels of apo CIII in plasma significantly affect the lipolytic but not the hepatic uptake pathways for metabolism of VLDL. FFA indicates free fatty acids.

apo CIII (1.8±0.5 pools/d) and apo CII (2.3±0.9 pool/d) were also similar but, in contrast to the CC group, the PRs of apo CIII (4.9±1.7 mg/kg per day) and apo CII (4.2±1.2 mg/kg per day) in the CT group were also similar, reflecting the reduced rate of synthesis of apo CIII in the CT versus the CC subjects, resulting from the *APOC3* R19X null mutation. For both apo CIII and apo CII, neither the FCR nor the PR was significantly different between the CT and CC subjects.

Discussion

We used stable isotopes to investigate, in vivo, the effects of heterozygosity for the *APOC3* R19X null mutation and the associated 50% lower levels of plasma apo CIII on apoB100 and TG metabolism. Our purpose was to determine the relative importance of haplodeficiency for 3 proposed physiological roles of apo CIII in VLDL metabolism: (1) inhibition of LpL-mediated lipolysis of VLDL-TG, (2) inhibition of hepatic uptake of VLDL or TG-rich remnant particles, and (3) stimulation of the incorporation of TG into VLDL in the liver. Each of these roles, which have been demonstrated by in vitro assays or by studies in cells and mouse models, could have a significant impact on the efficacy of therapeutic agents that partially inhibit apo CIII synthesis for treatment of hypertriglyceridemia and for prevention of CVD. The most significant differences we observed between affected CT and unaffected CC sib-pairs were the higher in FCRs for VLDL-TG and VLDL-apoB100 in the CT. The 50% lower circulating apo CIII levels in the R19X carriers resulted from the doubling of the rate of clearance of TG-rich apoB100-lipoproteins from the bloodstream. The clearance of VLDL occurs as a 2-stage process. First, LpL hydrolyzes the TG in nascent VLDL as these particles circulate through the vascular beds of adipose tissue and muscle,[35] generating what is typically designated as a remnant that still contains less TG, but enough to keep its density <1.006 (within the VLDL range). These particles circulate back to the liver, which is believed to be the site of the second stage. The latter actually has 2 components; 1 comprises additional lipolysis of remnant TG by LpL, and probably more importantly hepatic lipase, resulting in conversion of VLDL to IDL and LDL.[36] The second component involves interaction of apoB100 with one or more receptors and proteoglycans on the cell surface of the liver, resulting in internalization of the remnant particle.[37–39] Importantly, it is not clear whether these are completely independent parallel pathways or if some additional lipolysis is necessary before remnant uptake by the liver occurs. Our kinetic data do not allow us to differentiate between these possibilities and, therefore, we have made them independent, parallel pathways.

Our compartmental modeling of stable isotope enrichments of TG and apoB100 in VLDL, and of apoB100 in IDL and LDL, enabled us to determine the effect of partial loss of apo CIII on each of the second stage pathways. Indeed, our results indicate that the rates of conversion of VLDL particles to LDL were significantly greater in the carriers of R19X. The findings that the FCR of IDL-apoB100 (which was not removed directly) was also significantly greater in the CT group supports higher rates of conversion of VLDL to LDL, as does a nonsignificant, numerical increase in the PR of LDL-apoB100. All of the present results are in accord with those from our previous study in 2 sisters with complete loss of apo CIII because of a large homozygous chromosomal deletion, where we demonstrated marked increases in FCRs of VLDL-apoB100 and VLDL-TG as well as normal or increased rates of conversion of VLDL-apoB100 to LDL.[7] In

Table 4. Effects of *APOC3* R19X Mutation on Individual and Mean Fractional Rates of VLDL-ApoB100 Clearance, Conversion of VLDL-ApoB100 to LDL, and Direct Removal of VLDL-ApoB100

Participant	VLDL-ApoB100 FCR, pools/d	Conversion of VLDL-ApoB100 to LDL FCR, pools/d	Conversion of VLDL-ApoB100 to LDL Directly FCR, pools/d	Conversion of VLDL-ApoB100 to LDL Via IDL FCR, pools/d	Direct Removal of VLDL-ApoB100 FCR, pools/d
CC1	18.7	18.7	7.24	11.42	0.00
CC2	7.82	2.15	1.64	0.51	5.67
CC3	10.1	3.73	0.9	2.83	6.32
CC4	22.4	15.2	14.66	0.57	7.16
CC5	16.1	4.98	3.04	1.94	11.1
Mean±SD	15.0±6.0	9.0±7.5	5.5±5.7	3.5±4.6	6.05±3.98
CT1	37.8	37.8	28.43	9.32	0.00
CT2	20.5	11.9	5.22	6.7	8.56
CT3	22.8	22.8	11.41	11.41	0.00
CT4	40.3	22.0	19.14	2.83	18.3
CT5	33.5	11.9	9.90	2.05	21.5
Mean±SD	31.0±8.87	21.3±10.6	14.8±9.1	6.5±4.0	9.67±10.0
Difference±SD	16.0±3.02†	12.3±6.28*	9.3±7.2*	3.0±4.4	3.6±7.3

Participants are defined as CC (unaffected siblings), CT (affected siblings), in order of sib-pairs. VLDL: very-low-density lipoprotein, Data derived from compartmental modeling of stable isotope enrichments obtained over a 24 h. period. Data are presented as means and SD of the absolute FCRs of VLDL-apoB100, the conversion of VLDL-apoB100, and the direct removal of VLDL-apoB100, as well as the means and SD of the absolute differences in each parameter between the CT and CC groups. Conversion of VLDL-apoB100 to LDL includes both conversion of VLDL directly to IDL as well as VLDL to IDL and IDL to LDL. There was no direct conversion of VLDL to LDL required to fit the kinetic data; all the conversion occurred through IDL. Statistical significance assessed using paired *t* tests. ApoB indicates apolipoprotein B100; FCR, fractional clearance rate; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; PR: production rate; TG, triglycerides; and VLDL, very low-density lipoprotein.

*Significant $P<0.05$.

†Significant $P<0.001$.

the present study, we did not find a significant effect of partial loss of apo CIII synthesis on the uptake of VLDL remnants by the liver. This result might seem to conflict with results of recent studies of the efficacy of an apo CIII antisense in patients with no LpL, where the significant reductions in plasma TG levels observed would have required increased direct removal of TG-rich lipoproteins, presumably by the liver.[40] However, whereas hepatic removal of TG-rich lipoproteins was the only pathway that would be susceptible for inhibition by apo CIII in patients lacking LpL, our participants would have both of the apo CIII-susceptible pathways available for participation in the metabolism of VLDL. A simple explanation for our results might be that more efficient LpL-mediated lipolysis of nascent VLDL resulted in smaller VLDL that were more depleted of TG than post-lipolysis VLDL in nonaffected individuals and, therefore, more likely to be converted to IDL and LDL by additional lipolytic activities during the second stage of VLDL metabolism than to be internalized by LDL family receptor-mediated pathways present in the liver.[38,39] Our results also contrast with recent studies of the effects of apo CIII antisense in mice with targeted deletion of LpL, LDL receptors, the LDL receptor-related protein-1, and heparin sulfate proteoglycan receptors.[41] Their results led the authors to conclude that apo CIII inhibited hepatic uptake of remnant lipoproteins, but had little or no effect on LpL-mediated lipolysis of TG. Although those studies were convincing, they were in mice, where apoB48-apoE enriched lipoproteins are predominant, and where hepatic removal of remnants is much greater than in humans. Direct comparisons between the 2 studies are, therefore, very limited.

In contrast to the significant effect of reduced apo CIII on clearance of VLDL-TG and VLDL-apoB100, we did not find any evidence supporting a role for apo CIII in the incorporation of TG into nascent VLDL within the liver.[10,12] Our result agrees with data showing that treatment of apo CIII transgenic mice with an apo CIII antisense had no effect on rates of VLDL secretion,[42] and with our previous finding of normal VLDL-apoB100 and VLDL-TG PRs in the 2 patients with complete absence of apo CIII.[7] Our results, indicating that partial loss of apo CIII has a significant impact on the lipolytic pathway but not on direct removal of VLDL remnants, raise obvious questions about the potential of therapies that lower apo CIII levels. On the one hand, it is clear that the marked increase in VLDL-TG FCR is consistent

Table 5. Effects of *APOC3* R19X Mutation on Kinetic Parameters for Apo CIII and Apo CII Metabolism

		CC (±SD)	CT (±SD)	Difference
Apo CIII	FCR	1.3±0.7	1.8±0.5	0.59±0.38
	PR	7.4±6	4.9±2	−2.54±4.83
Apo CII	FCR	1.4±0.8	2.3±1	0.90±0.74
	PR	3.9±2	4.2±1	0.33±1.34

Participants are defined as CC (unaffected siblings) and CT (affected siblings). Data are presented as means and SD. There were no significant differences for any of the variables between CC and CT groups or apo CIII and apo CII. FCR indicates fractional clearance rate; and PR, production rate.

with the dramatic reductions in TG levels observed in a study where patients with marked hypertriglyceridemia without mutations in LpL were treated with an antisense to apo CIII.[43] Those results indicate the utility of apo CIII-lowering therapy to prevent pancreatitis in such patients. In contrast, if therapies that reduce apo CIII synthesis result in higher rates of conversion of VLDL remnants to LDL with no change or modest increases in LDL-cholesterol or apoB100 levels,[43,44] how could that translate to risk for CVD? Specifically, how do our findings relate to the decreased levels of coronary calcification in R19X carriers[17] and very significant reductions in CVD risk observed in cohorts with loss of function variants in the *APOC3* gene.[15,16] A simple answer is that reducing the number of VLDL remnants, which carry more cholesterol per particle than LDL, is beneficial regardless of the mechanism involved. Individuals with dyslipidemia and the apo E2/2 genotype accumulate both apoB100 and apoB48 remnants and have significantly increased risk for CVD.[45,46] Amish individuals heterozygous for the *APOC3* R19X mutation and individuals with the combined deletion of *APOA1/APOC3/APOA4/APOA5* had decreased postprandial TG levels,[7,47] which have been shown to be atherogenic.[48,49] More dramatic reductions in postprandial lipid levels were reported recently in a small number of individuals homozygous for the R19X mutation in *APOC3*.[50] Of note, individuals in that study who were either heterozygotes or homozygoutes for R19X had LDL-cholesterol levels that were similar to noncarriers.[49] A recently published study comparing the roles of remnant cholesterol versus LDL-cholesterol in reduced CVD risk in 137 895 individuals with loss of function of *APOC3* found that lower concentrations of remnant cholesterol accounted for nearly all of the benefit of lower apo CIII levels.[51] Importantly, genetic studies have demonstrated that gain of function variants in the *LPL* gene are associated with reduced risk for CVD, whereas loss of function variants in this gene are associated with increased risk.[52,53] Our stable isotope kinetic studies of apo CIII and apo CII showed the expected lower apo CIII PR in the CT compared with the CC group, but no difference in apo CII PR between affected and unaffected siblings. These results are in accord with the isolated R19X mutation in the CT group and indicative of independent regulation of the expression of the *APOC3* and *APOC2* genes which are on different chromosomes. In contrast, the FCRs of both apo CIII and apo CII were greater in the affected compared with the unaffected siblings, suggestive of a common pathway for the clearance of these 2 apolipoproteins from the plasma, possibly along with VLDL remnants. The FCRs of apo CIII and apo CII are greater than those of both LDL-apoB100 and HDL apo AI, and slower than the FCRs of VLDL- and IDL-apoB100. However, as both apo CIII and apo CII are associated with all the major lipoproteins, a model that has reservoirs of apo CIII and apo CII on HDL that would feed a catabolic pathway through VLDL remnants seems reasonable to explain our results. A recent article describing the effects of a rare mutation in *APOC3* that affected the binding of the apolipoprotein to lipoproteins resulted in clearance of apo CIII via the kidney.[54] We previously demonstrated a role of the kidney in the catabolism of apo AI in hypertriglyceridemic individuals.[55] While we cannot rule out involvement of the kidney in the increased catabolism of apo CIII and apo CII in the CT group, evidence for a significant pool of free apo CIII or free apo CII in plasma is lacking,[56,57] and the mutation in *APOC3* in our CT subjects results in decreased secretion with no evidence of altered lipoprotein distribution of apo CIII synthesized by the normal allele. Of note, the lower levels of apo CII in the CT group did not seem to restrict or limit the effect of low apo CIII on VLDL-TG clearance. This is consistent with the lack of any alteration in plasma lipid levels in heterozygotes for apo CII deficiency.[58] Despite uncertainties about the effects on CVD risk, which will require large randomized, placebo-controlled CVD outcome trials to resolve, the very large reductions of plasma TG levels observed with apo CIII antisense indicate that therapies to significantly lower apo CIII concentrations in the circulation could add a new, potent approach for the prevention of pancreatitis in patients with severe hypertriglyceridemia.[59] We also realize that other proatherogenic or proinflammatory effects of apo CIII have been reported, and these may also be reversed by therapies that reduce apo CIII levels.[60,61]

Study Limitations

This study included a small number of participants, a shortcoming that we attempted to alleviate by using sib-pairs. We also did not address the heterogeneous nature of apoB100-lipoproteins, particularly related to the presence of apo CIII on only a portion of VLDL, IDL, and LDL, demonstrated first by Alaupovic et al[62] and then in a series of stable isotope kinetic studies by Zheng et al,[63] Mendivil et al,[64] and Sacks et al.[65] The finding that there are apoB100-lipoproteins with and without apo CIII could have an important implication for the present study. We would note, however, that Sacks et al[65] have reported that 40% to 80% of VLDL from individuals with normal TG levels contain 60 to 100 apo CIII molecules per particle. Thus, it seems likely that a 50% reduction of apo CIII in R19X carriers would not significantly alter the proportion of VLDL that containing significant numbers of apo CIII molecules, and that the affected and unaffected siblings would have relatively similar proportions of VLDL with and without apo CIII. Our finding that the proportions of both apo CIII and apo CII in VLDL were similar in affected and unaffected siblings suggests similar proportions of apo CIII-containing VLDL in both groups. Importantly, we acknowledge that the metabolic differences we are reporting between affected and unaffected siblings may not completely mimic the effects of a therapeutic agent that reduces levels of plasma apo CIII.

Conclusions

The present studies demonstrate the physiological effects of 50% reductions in plasma apo CIII resulting from heterozygosity for a naturally occurring loss of function mutation in the *APOC3* gene. Lower apo CIII levels in the circulation resulted in higher rates of lipolysis of VLDL-TG and higher rates of conversion of VLDL to LDL in the affected siblings. We did not observe changes in the rate of direct remnant removal of VLDL remnants by the liver or in rates of secretion of VLDL. Our results, together with those from cohorts with loss of function variants in the *APOCIII* gene, provide evidence for the increased atherogenicity of VLDL (and chylomicron) remnants, as well as support for therapies that would reduce remnant concentrations regardless of the mechanism

involved. The impact of loss of function variants in apo CIII might be of particular importance in the postprandial period.

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Disclosures

The authors do not have relationships that are in conflict with the current article.

References

1. Brown WV, Levy RI, Fredrickson DS. Studies of the proteins in human plasma very low density lipoproteins. *J Biol Chem*. 1969;244:5687–5694.
2. Brown WV, Baginsky ML. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem Biophys Res Commun*. 1972;46:375–382.
3. LaRosa JC, Levy RI, Herbert P, Lux SE, Fredrickson DS. A specific apoprotein activator for lipoprotein lipase. *Biochem Biophys Res Commun*. 1970;41:57–62.
4. Windler E, Chao Y, Havel RJ. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effects of homologous apolipoprotein E and individual C apoproteins. *J Biol Chem*. 1980;255:8303–8307.
5. Quarfordt SH, Michalopoulos G, Schirmer B. The effect of human C apolipoproteins on the *in vitro* hepatic metabolism of triglyceride emulsions in the rat. *J Biol Chem*. 1982;257:14642–14647.
6. Norum RA, Lakier JB, Goldstein S, Angel A, Goldberg RB, Block WD, Noffze DK, Dolphin PJ, Edelglass J, Bogorad DD, Alaupovic P. Familial deficiency of apolipoproteins A-I and C-III and precocious coronary-artery disease. *N Engl J Med*. 1982;306:1513–1519. doi: 10.1056/NEJM198206243062503
7. Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R, Brown WV. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J Clin Invest*. 1986;78:1287–1295. doi: 10.1172/JCI112713
8. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science*. 1990;249:790–793.
9. de Silva HV, Lauer SJ, Wang J, Simonet WS, Weisgraber KH, Mahley RW, Taylor JM. Overexpression of human apolipoprotein C-III in transgenic mice results in an accumulation of apolipoprotein B48 remnants that is corrected by excess apolipoprotein E. *J Biol Chem*. 1994;269:2324–2335.
10. Aalto-Setälä K, Fisher EA, Chen X, Chajek-Shaul T, Hayek T, Zechner R, Walsh A, Ramakrishnan R, Ginsberg HN, Breslow JL. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. *J Clin Invest*. 1992;90:1889–1900. doi: 10.1172/JCI116066
11. Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J Biol Chem*. 1994;269:23610–23616.
12. Sundaram M, Zhong S, Bou Khalil M, Links PH, Zhao Y, Iqbal J, Hussain MM, Parks RJ, Wang Y, Yao Z. Expression of apolipoprotein C-III in McA-RH7777 cells enhances VLDL assembly and secretion under lipid-rich conditions. *J Lipid Res*. 2010;51:150–161. doi: 10.1194/M900346-JLR200
13. Hulley SB, Rosenman RH, Bawol RD, Brand RJ. Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease. *N Engl J Med*. 1980;302:1383–1389. doi: 10.1056/NEJM198006193022503
14. Chapman MJ, Ginsberg HN, Amarenco P, et al; European Atherosclerosis Society Consensus Panel. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J*. 2011;32:1345–1361. doi: 10.1093/eurheartj/ehr112
15. Musunuru K, Kathiresan S. Surprises from genetic analyses of lipid risk factors for atherosclerosis. *Circ Res*. 2016;118:579–585. doi: 10.1161/CIRCRESAHA.115.306398
16. Pare G, Anand SS. Mendelian randomisation, triglycerides, and CHD. *Lancet*. 2010;375:1584–1586. doi: 10.1016/S0140-6736(10)60659-9
17. Pollin TI, Damcott CM, Shen H, Ott SH, Shelton J, Horenstein RB, Post W, McLenithan JC, Bielak LF, Peyser PA, Mitchell BD, Miller M, O'Connell JR, Shuldiner AR. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science*. 2008;322:1702–1705. doi: 10.1126/science.1161524
18. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32–41. doi: 10.1056/NEJMoa1308027
19. Crosby J, Peloso GM, Auer PL, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med*. 2014;371:22–31.
20. Gaudet D, Drouin-Chartier JP, Couture P. Lipid metabolism and emerging targets for lipid-lowering therapy. *Can J Cardiol*. 2017;33:872–882. doi: 10.1016/j.cjca.2016.12.019
21. Bauer RC, Khetarpal SA, Hand NJ, Rader DJ. Therapeutic targets of triglyceride metabolism as informed by human genetics. *Trends Mol Med*. 2016;22:328–340. doi: 10.1016/j.molmed.2016.02.005
22. Reyes-Soffer G, Pavlyha M, Ngai C, et al. Effects of PCSK9 inhibition with alirocumab on lipoprotein metabolism in healthy humans. *Circulation*. 2017;135:352–362. doi: 10.1161/CIRCULATIONAHA.116.025253
23. Ramakrishnan R. Studying apolipoprotein turnover with stable isotope tracers - correct analysis is by modeling enrichments. *Jour Lipid Research*. 2006;47:2738–2753.
24. Ramakrishnan R, Ramakrishnan JD. Using mass measurements in tracer studies–a systematic approach to efficient modeling. *Metabolism*. 2008;57:1078–1087. doi: 10.1016/j.metabol.2008.03.011
25. Nagashima K, Lopez C, Donovan D, Ngai C, Fontanez N, Bensadoun A, Fruchart-Najib J, Holleran S, Cohn JS, Ramakrishnan R, Ginsberg HN. Effects of the PPARgamma agonist pioglitazone on lipoprotein metabolism in patients with type 2 diabetes mellitus. *J Clin Invest*. 2005;115:1323–1332. doi: 10.1172/JCI23219
26. Pan Y, Zhou H, Mahsut A, et al. Static and turnover kinetic measurement of protein biomarkers involved in triglyceride metabolism including apoB48 and apoA5 by LC/MS/MS. *J Lipid Res*. 2014;55:1179–1187. doi: 10.1194/jlr.D047829
27. Ginsberg HN, Ramakrishnan R. Kinetic studies of the metabolism of rapidly exchangeable apolipoproteins may leave investigators and readers with exchangeable results. *Arterioscler Thromb Vasc Biol*. 2008;28:1685–1686. doi: 10.1161/ATVBAHA.108.174185
28. Lassman ME, McAvoy T, Lee A, Chappell DA, Wong O, Zhou H, Reyes-Soffer G, Ginsberg HN, Millar JS, Rader DJ, Gutstein DE, Laterza O. Practical immunoaffinity-enrichment LC-MS for measuring protein kinetics of low-abundance proteins. *Clin Chem*. 2014;60:1217–1224.
29. Havel RJ, Kane JP, Kashyap ML. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J Clin Invest*. 1973;52:32–38. doi: 10.1172/JCI107171
30. Huff MW, Fidge NH, Nestel PJ, Billington T, Watson B. Metabolism of C-apolipoproteins: kinetics of C-II, C-III1 and C-III2, and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects. *J Lipid Res*. 1981;22:1235–1246.
31. Chan DC, Watts GF, Ooi EM, Ji J, Johnson AG, Barrett PH. Atorvastatin and fenofibrate have comparable effects on VLDL-apolipoprotein C-III kinetics in men with the metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2008;28:1831–1837. doi: 10.1161/ATVBAHA.108.170530
32. Ooi EM, Chan DT, Watts GF, Chan DC, Ng TW, Dogra GK, Irish AB, Barrett PH. Plasma apolipoprotein C-III metabolism in patients with chronic kidney disease. *J Lipid Res*. 2011;52:794–800. doi: 10.1194/jlr.M011163

33. Sacks FM, Zheng C, Cohn JS. Complexities of plasma apolipoprotein C-III metabolism. *J Lipid Res*. 2011;52:1067–1070. doi: 10.1194/jlr.E015701
34. Huff MW, Nestel PJ. Metabolism of apolipoproteins CII, CIII1, CIII2 and VLDL-B in human subjects consuming high carbohydrate diets. *Metabolism*. 1982;31:493–498.
35. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. *Am J Physiol Endocrinol Metab*. 2009;297:E271–E288. doi: 10.1152/ajpendo.90920.2008
36. Kobayashi J, Miyashita K, Nakajima K, Mabuchi H. Hepatic lipase: a comprehensive view of its role on plasma lipid and lipoprotein metabolism. *J Atheroscler Thromb*. 2015;22:1001–1011. doi: 10.5551/jat.31617
37. Foley EM, Esko JD. Hepatic heparan sulfate proteoglycans and endocytic clearance of triglyceride-rich lipoproteins. *Prog Mol Biol Transl Sci*. 2010;93:213–233. doi: 10.1016/S1877-1173(10)93010-X
38. Ishibashi S, Perrey S, Chen Z, Osuga Ji, Shimada M, Ohashi K, Harada K, Yazaki Y, Yamada N. Role of the low density lipoprotein (LDL) receptor pathway in the metabolism of chylomicron remnants. A quantitative study in knockout mice lacking the LDL receptor, apolipoprotein E, or both. *J Biol Chem*. 1996;271:22422–22427.
39. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest*. 1998;101:689–695. doi: 10.1172/JCI1240
40. Gaudet D, Brisson D, Tremblay K, Alexander VJ, Singleton W, Hughes SG, Geary RS, Baker BF, Graham MJ, Crooke RM, Witztum JL. Targeting APOC3 in the familial chylomicronemia syndrome. *N Engl J Med*. 2014;371:2200–2206. doi: 10.1056/NEJMoa1400284
41. Gordts PL, Nock R, Son NH, Ramms B, Lew I, Gonzales JC, Thacker BE, Basu D, Lee RG, Mullick AE, Graham MJ, Goldberg IJ, Crooke RM, Witztum JL, Esko JD. ApoC-III inhibits clearance of triglyceride-rich lipoproteins through LDL family receptors. *J Clin Invest*. 2016;126:2855–2866. doi: 10.1172/JCI86610
42. Graham MJ, Lee RG, Bell TA III, Fu W, Mullick AE, Alexander VJ, Singleton W, Viney N, Geary R, Su J, Baker BF, Burkey J, Crooke ST, Crooke RM. Antisense oligonucleotide inhibition of apolipoprotein C-III reduces plasma triglycerides in rodents, nonhuman primates, and humans. *Circ Res*. 2013;112:1479–1490. doi: 10.1161/CIRCRESAHA.111.300367
43. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, Geary RS, Hughes SG, Viney NJ, Graham MJ, Crooke RM, Witztum JL, Brunzell JD, Kastelein JJ. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med*. 2015;373:438–447. doi: 10.1056/NEJMoa1400283
44. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, Oberhollenzer F, Egger G, Witztum JL, Alexander VJ, Willeit J, Kiechl S, Mayr M. Very-low-density lipoprotein-associated apolipoproteins predict cardiovascular events and are lowered by inhibition of APOC-III. *J Am Coll Cardiol*. 2017;69:789–800. doi: 10.1016/j.jacc.2016.11.065
45. Gregg RE, Zech LA, Schaefer EJ, Brewer HB Jr. Type III hyperlipoproteinemia: defective metabolism of an abnormal apolipoprotein E. *Science*. 1981;211:584–586.
46. CB B. Type III hyperlipoproteinemia: still worth considering? *Prog Cardiovasc Dis*. 2016;59:119–124.
47. Norum RA, Forte TM, Alaupovic P, Ginsberg HN. Clinical syndrome and lipid metabolism in hereditary deficiency of apolipoproteins A-I and C-III, variant I. In: A. Angel and J. Frohlich, eds. *Lipoprotein Deficiency Syndromes*: Plenum Publishing; 1986.
48. Langsted A, Freiberg JJ, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Nordestgaard BG. Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med*. 2011;270:65–75. doi: 10.1111/j.1365-2796.2010.02333.x
49. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation*. 2008;118:993–1001. doi: 10.1161/CIRCULATIONAHA.108.777334
50. Saleheen D, Natarajan P, Armean IM, et al. Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature*. 2017;544:235–239. doi: 10.1038/nature22034
51. Wulff AB, Nordestgaard BG, Tybjærg-Hansen A. APOC3 loss-of-function mutations, remnant cholesterol, low-density lipoprotein cholesterol, and cardiovascular risk: mediation- and meta-analyses of 137 895 individuals. *Arterioscler Thromb Vasc Biol*. 2018;38:660–668. doi: 10.1161/ATVBAHA.117.310473
52. Rip J, Nierman MC, Ross CJ, Jukema JW, Hayden MR, Kastelein JJ, Stroes ES, Kuivenhoven JA. Lipoprotein lipase S447X: a naturally occurring gain-of-function mutation. *Arterioscler Thromb Vasc Biol*. 2006;26:1236–1245. doi: 10.1161/01.ATV.0000219283.10832.43
53. Stirrups KE, Masca NG, Erdmann J, et al; Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators SN, . Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary disease. *N Engl J Med*. 2016;374:1134–44.
54. Khetarpal SA, Zeng X, Millar JS, et al. A human APOC3 missense variant and monoclonal antibody accelerate apoC-III clearance and lower triglyceride-rich lipoprotein levels. *Nat Med*. 2017;23:1086–1094. doi: 10.1038/nm.4390
55. Horowitz BS, Goldberg IJ, Merab J, Vanni TM, Ramakrishnan R, Ginsberg HN. Increased plasma and renal clearance of an exchangeable pool of apolipoprotein A-I in subjects with low levels of high density lipoprotein cholesterol. *J Clin Invest*. 1993;91:1743–1752. doi: 10.1172/JCI116384
56. Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R, Brown WV. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J Clin Invest*. 1986;78:1287–1295. doi: 10.1172/JCI112713
57. Gibson JC, Rubinstein A, Brown WV, Ginsberg HN, Greten H, Norum R, Kayden H. Apo E-containing lipoproteins in low or high density lipoprotein deficiency. *Arteriosclerosis*. 1985;5:371–380.
58. Gabelli C, Bilato C, Santamarina-Fojo S, Martini S, Brewer HB Jr, Crepaldi G, Baggio G. Heterozygous apolipoprotein C-II deficiency: lipoprotein and apoprotein phenotype and RsaI restriction enzyme polymorphism in the Apo C-IIPadova kindred. *Eur J Clin Invest*. 1993;23:522–528.
59. Brunzell JD, Schrott HG. The interaction of familial and secondary causes of hypertriglyceridemia: role in pancreatitis. *J Clin Lipidol*. 2012;6:409–412. doi: 10.1016/j.jacl.2012.06.005
60. Jensen MK, Rimm EB, Furtado JD, Sacks FM. Apolipoprotein C-III as a potential modulator of the association between HDL-cholesterol and incident coronary heart disease. *J Am Heart Assoc*. 2012;1:jah3-e000232.
61. Mendivil CO, Rimm EB, Furtado J, Chiuve SE, Sacks FM. Low-density lipoproteins containing apolipoprotein C-III and the risk of coronary heart disease. *Circulation*. 2011;124:2065–2072. doi: 10.1161/CIRCULATIONAHA.111.056986
62. Alaupovic P, McConathy WJ, Fesmire J, Tavella M, Bard JM. Profiles of apolipoproteins and apolipoprotein B-containing lipoprotein particles in dyslipoproteinemias. *Clin Chem*. 1988;34(8B):B13–B27.
63. Zheng C, Khoo C, Ikewaki K, Sacks FM. Rapid turnover of apolipoprotein C-III-containing triglyceride-rich lipoproteins contributing to the formation of LDL subfractions. *J Lipid Res*. 2007;48:1190–1203. doi: 10.1194/jlr.P600011-JLR200
64. Mendivil CO, Zheng C, Furtado J, Lel J, Sacks FM. Metabolism of very-low-density lipoprotein and low-density lipoprotein containing apolipoprotein C-III and not other small apolipoproteins. *Arterioscler Thromb Vasc Biol*. 2010;30:239–245. doi: 10.1161/ATVBAHA.109.197830
65. Sacks FM. The crucial roles of apolipoproteins E and C-III in apoB lipoprotein metabolism in normolipidemia and hypertriglyceridemia. *Curr Opin Lipidol*. 2015;26:56–63.

Highlights

- *APOC3* R19X null mutation causes 50% lower levels of plasma apo CIII.
- The changes in apo CIII levels were because of a doubling of the rate of clearance of TG-rich apoB100-lipoproteins from the bloodstream.
- These results provide strong evidence that therapies which increase the efficiency of conversion of VLDL to LDL, thereby reducing remnant concentrations, should reduce the risk of cardiovascular disease.