Targets of Statin Therapy:
LDL Cholesterol, Non-HDL Cholesterol, and Apolipoprotein B in Type 2 Diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS)

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BACKGROUND: LDL can vary considerably in its cholesterol content; thus, lowering LDL cholesterol (LDLC) as a goal of statin treatment implies the existence of considerable variation in the extent to which statin treatment removes circulating LDL particles. This consideration is particularly applicable in diabetes mellitus, in which LDL is frequently depleted of cholesterol.

METHODS: Type 2 diabetes patients randomly allocated to 10 mg/day atorvastatin (n = 1154) or to placebo (n = 1196) for 1 year were studied to compare spontaneous and statin-induced apolipoprotein B (apo B) concentrations (a measure of LDL particle concentration) at LDLC and non-HDL cholesterol (non-HDLC) concentrations proposed as statin targets in type 2 diabetes.

RESULTS: Patients treated with atorvastatin produced lower serum apo B concentrations at any given LDLC concentration than patients on placebo. An LDLC concentration of 1.8 mmol/L (70 mg/dL) during atorvastatin treatment was equivalent to a non-HDLC concentration of 2.59 mmol/L (100 mg/dL) or an apo B concentration of 0.8 g/L. At the more conservative LDLC targets of 2.59 mmol/L (100 mg/dL) and 3.37 mmol/L (130 mg/dL) for non-HDLC, however, the apo B concentration exceeded the 0.9-g/L value anticipated in the recent Consensus Statement from the American Diabetes Association and the American College of Cardiology.

CONCLUSIONS: The apo B concentration provides a more consistent goal for statin treatment than the LDLC or non-HDLC concentration.

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Statin drugs have proved effective for both primary and secondary prevention of cardiovascular disease (CVD)[10] in nondiabetic and diabetic populations (1, 2). Their primary mode of action is to decrease the circulating LDL concentration by up-regulation of hepatic LDL receptor–mediated catabolism in response to the competitive inhibition of hepatic cholesterol biosynthesis at the level of hydroxymethylglutaryl-CoA reductase (3). Circulating LDL is not believed to participate directly in atherogenesis. It must first undergo some modification that affects the structure of its apolipoprotein B100 (apo B) so that it becomes a ligand for the scavenger receptors of monocyte macrophages in the arterial wall (4). Cholesterol then accumulates in the macrophage cytoplasm to form the foam cells characteristic of fatty streaks and advanced atheromatous lesions. It is apo B, however, not the cholesterol component of LDL, that facilitates LDL uptake by macrophages. The issue, then, is whether apo B or LDL cholesterol (LDLC) is the most suitable target of statin therapy. Two observations have further stimulated the debate. First, LDLC may not provide as good an estimate of the concentration of LDL particles, particularly when triglycerides are also increased, because of the

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[10] Nonstandard abbreviations: CVD, cardiovascular disease; apo B, apolipoprotein B; LDLC, LDL cholesterol; HDLC, HDL cholesterol; CARDS, Collaborative Atorvastatin Diabetes Study; RIQAS, Randox International Quality Assessment Scheme; ATPIII, Adult Treatment Panel III.

presence of small cholesterol-depleted LDL particles (5–7). Second, statin trials in which both LDLC and apo B responses have been measured have demonstrated that the percent decrease in LDLC concentration is greater than for apo B (8).

Considerable controversy surrounds the proposal that apo B may provide a better means of predicting CVD risk than LDLC (9). Extensive investigation has revealed that apo B generally is the better predictor, but the practical advantage may be small (10–12). On the other hand, the question of whether LDLC or apo B provides the better target of statin treatment has not been explored as much as it deserves. If considerable individual variation in LDL particle concentration were demonstrated at the LDLC concentrations currently recommended as therapeutic targets for statins, some patients might be left with a CVD risk that would have been further diminished if apo B instead had been the target of treatment (9). Non-HDL cholesterol (non-HDLC) has also been proposed as a possible statin target. It has been suggested to be a better reflection of apo B–containing lipoprotein concentrations than LDLC and to obviate the introduction of apo B measurement more widely in patient management (13); however, this proposal has been disputed (14). Furthermore, non-HDLC is generally used in the clinic as a therapeutic target only when triglyceride concentrations are raised, precluding the calculation of LDLC concentrations via the Friedewald formula (15). In the present report, we evaluate LDLC, non-HDLC, and apo B as potential statin targets in a large trial of atorvastatin for treating type 2 diabetes. The issue is particularly relevant in this trial because of the known prevalence of cholesterol-depleted LDL in this disease (16, 17).

Materials and Methods

STUDY POPULATION
The Collaborative Atorvastatin Diabetes Study (CARDS) was a double-blind, randomized, placebo-controlled, multicenter trial of atorvastatin (10 mg/day) for the primary prevention of CVD in type 2 diabetes (18–20). The study received ethical approval both centrally and at each participating institution, and each patient gave written informed consent. The study included 2838 randomized patients (68% men) between 40 and 75 years of age who took at least 1 dose of the study drug. The primary endpoint of the trial was the first acute coronary heart disease event (myocardial infarction, hospitalized unstable angina, acute coronary heart disease death), coronary revascularization procedure, or stroke. In addition, information about all causes of death was collected. To enter the trial, patients had to be free of macrovascular disease, to have serum LDLC concentrations ≤4.14 mmol/L (≤160 mg/dL) and to have fasting serum triglyceride concentrations ≤6.78 mmol/L (≤600 mg/dL). In addition, study participants were required to have at least one of the following cardiovascular risk factors: hypertension on treatment, a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg on 2 successive occasions, any retinopathy, proteinuria including microalbuminuria, or current smoking. The trial was terminated 2 years earlier than planned at the request of the Safety Committee because of the clear benefit of active treatment ($P < 0.001$, 2-tailed test) (19, 20). The median time of patient participation in the trial was 3.9 years. The most complete lipid and lipoprotein results after the initiation of treatment were at the first annual visit, when the study protocol required that apo B be measured for the first time since randomization.

LABORATORY METHODS
All participants were asked to fast from 10 PM the previous day. Cholesterol in serum and lipoproteins was measured with the CHOD-PAP method on a Cobas Mira analyzer (Roche Diagnostics). Triglycerides were measured with the GPO-PAP method (Roche Diagnostics), and serum apo B was measured by immunoturbidimetry (Roche Diagnostics) on the same instrument with calibration traceable to the IFCC primary standards (21). HDL was isolated by heparin–manganese precipitation of other serum lipoproteins (22). When serum triglycerides exceeded 4 mmol/L (354 mg/dL), VLDL was removed before the heparin–manganese procedure by ultracentrifugation for 18 h at 144 000g (Beckman L8–55; Beckman Coulter) at a density of 1.006 kg/L. The laboratory participated in the UK Randox International Quality Assessment Scheme (RIQAS) (Randox Laboratories). The HDLC method was aligned with the results of the CDC laboratory participating in this scheme.

The LDLC concentration was calculated with the Friedewald formula (23) when the serum triglyceride concentration was ≤4 mmol/L (≤354 mg/dL). When serum triglycerides exceeded 4 mmol/L, the LDLC concentration was obtained by subtracting the HDLC concentration from that in the D1.006-kg/L ultracentrifugation infranatant, which was obtained by tube slicing (24).

STATISTICAL METHODS
This study focuses on the 2350 individuals in CARDS (of 2838 participants) who had complete data at their first annual visit after randomization to treatment. The linear-regression equations for the correlations between LDLC and apo B concentrations and between non-HDLC and apo B concentrations were computed for measurements taken after 12 months of treatment for both the placebo and active atorvastatin arms.

Table 1. apo B concentrations at different LDLC concentrations in placebo-treated and atorvastatin-treated patients after 1 year of treatment.[a]

	Placebo-treated patients		Atorvastatin-treated patients	
LDLC	n	apo B, g/L	n	apo B, g/L
All	1154	1.105 (1.092–1.118)	1196	0.797 (0.785–0.808)
>4 mmol/L (>154 mg/dL)	150	1.341 (1.312–1.37)	13	1.24 (1.063–1.417)
>3–4 mmol/L (>116–154 mg/dL)	517	1.161 (1.142–1.176)	57	1.105 (1.061–1.149)
>2–3 mmol/L (>77–116 mg/dL)	367	0.992 (0.974–1.01)	381	0.908 (0.892–0.924)
≤2 mmol/L (≤77 mg/dL)	120	0.913 (0.867–0.960)	745	0.708 (0.697–0.720)

[a] apo B data are presented as the mean (95% CI). The mean LDLC concentration (95% CI) in study participants receiving placebo was 3.08 mmol/L (3.06–3.13 mmol/L), or 119 mg/dL (118–121 mg/dL). The mean LDLC concentration (95% CI) in study participants receiving atorvastatin was 1.86 mmol/L (1.81–1.89 mmol/L), or 72 mg/dL (70–73 mg/dL).

These equations were used to predict the apo B concentration equivalent to critical concentrations of LDLC and non-HDLC proposed in national and international recommendations for CVD prevention (15, 25, 26). All analyses were performed with SAS statistical software (version 8.12; SAS Institute) at a 2-sided significance level of 0.05.

Results

### CHANGES IN LIPIDS, LIPOPROTEINS, AND APOLIPOPROTEINS

Compared with placebo, atorvastatin treatment lowered the LDLC concentration by a mean of 40.9% (95% CI, 40.1%–41.6%), whereas atorvastatin treatment decreased the non-HDLC concentration by 38.1% (95% CI, 37.2%–39.0%) and the apo B concentration by 24.3% (95% CI, 23.4%–25.2%) (all $P < 0.0001$; see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue3). Active atorvastatin treatment increased the mean HDLC concentration by 1.6% (95% CI, 1.0%–2.1%; $P < 0.05$).

apo B AT STATIN-INDUCED AND SPONTANEOUSLY OCCURRING LDLC CONCENTRATIONS

When study participants on atorvastatin and on placebo were stratified by LDLC concentration, it was evident that the apo B concentration was lower in actively treated patients in any given LDLC concentration range (Table 1). This result was not explained by differences in median LDLC concentration between atorvastatin- and placebo-treated patients in each of these strata (i.e., LDLC >4 mmol/L, 4.28 vs 4.26 mmol/L, respectively; LDLC >3–4 mmol/L, 3.33 vs 3.44 mmol/L; LDLC >2–3 mmol/L, 2.31 vs 2.61 mmol/L; and LDLC ≤2 mmol/L, 1.52 vs 1.70 mmol/L).

SERUM apo B CONCENTRATIONS AT THERAPEUTIC TARGETS FOR LDLC AND NON-HDLC

Fig. 1 shows the relationships between serum apo B and LDLC concentrations at the end of 1 year of treatment with atorvastatin or placebo. There was a marked tendency for the apo B concentration to be higher at any given LDLC concentration in patients on placebo compared with those on atorvastatin, as predicted from Table 1. In the US, the Adult Treatment Panel III (ATPIII) LDLC targets for statin therapy (depending on risk and clinician preference) are 3.37 mmol/L (130 mg/dL), 2.59 mmol/L (100 mg/dL), and 1.813 mmol/L (70 mg/dL) (15). In Europe, an LDLC target of 2.59 mmol/L (100 mg/dL) is advocated (25). Table 2 shows that at these LDLC concentrations, higher serum apo B concentrations typically occurred spontaneously in patients on placebo, compared with those in patients who were assigned to atorvastatin treatment. In Britain, the authorities differ as to whether the LDLC target of statin treatment should be 3 mmol/L (116 mg/dL) or 2 mmol/L (77 mg/dL) (26). The apo B concentration was also lower at both of these LDLC concentrations achieved with atorvastatin therapy in type 2 diabetes than at similar LDLC concentrations occurring spontaneously in patients on placebo. Fig. 2 and Table 2 show that a similar disparity does not exist between non-HDLC and serum apo B concentrations in patients on atorvastatin compared with those on placebo. The table shows data for the non-HDLC concentrations of 2.59, 3.37, and 4.14 mmol/L (100, 130, and 160 mg/dL) because these concentrations are the targets recommended by the ATPIII for statin treatment in hypertriglyceridemia (15). At both an LDLC concentration of 2.59 mmol/L (100 mg/dL) and a non-HDLC concentration of 3.37 mmol/L (130 mg/dL), however, the concentration of apo B in patients on atorvastatin is higher than anticipated in the recent Consensus State-
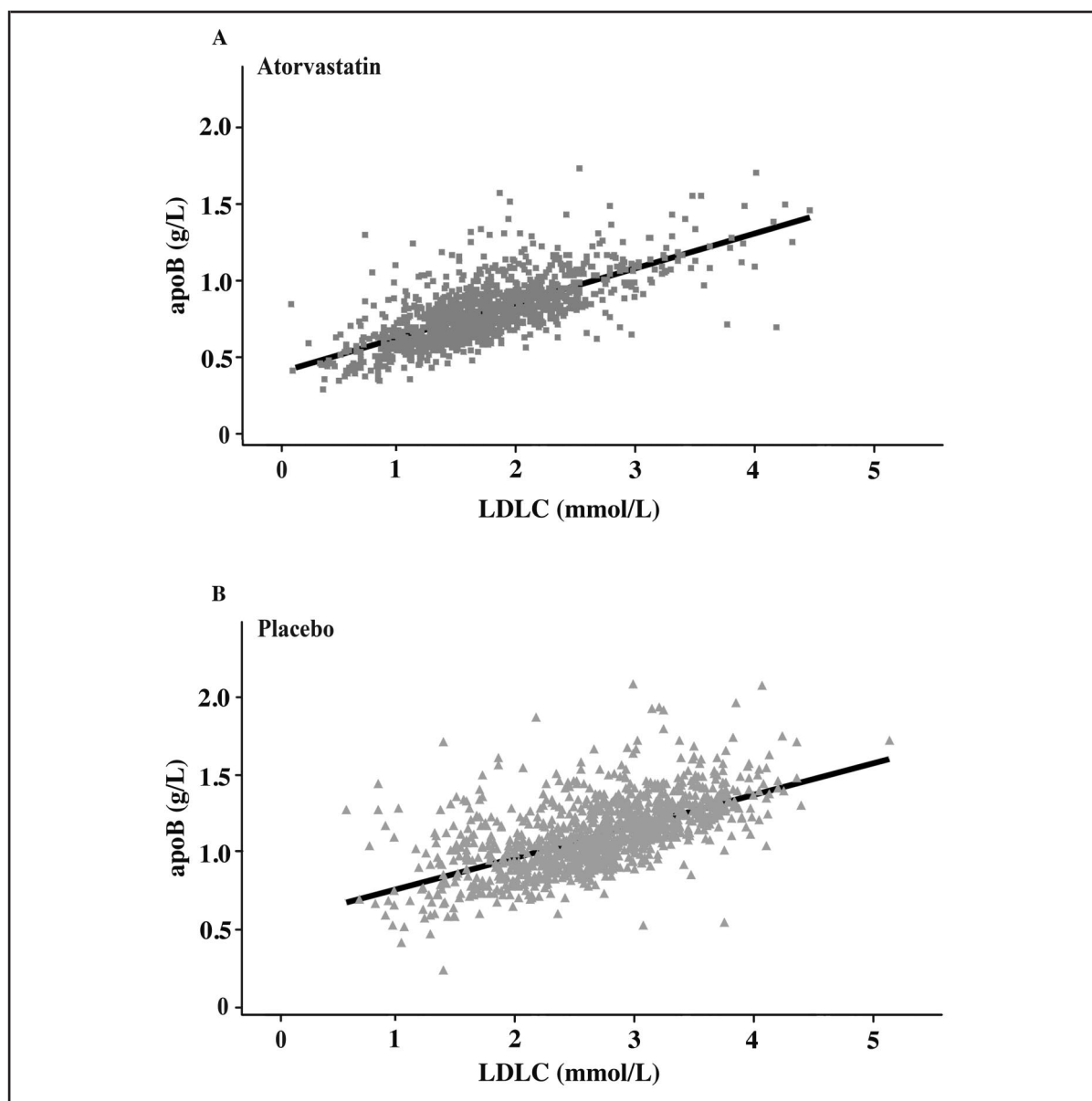
Fig. 1. Plots of serum apo B concentration against LDLC concentration after 1 year of treatment.

(A), After treatment with 10 mg/day atorvastatin: $y = 0.209x + 0.407$ g/L, where y is the apo B concentration and x is the LDLC concentration [SE of the slope (95% CI), 0.006 (0.197−0.221); $r^2 = 49.50\%$; $P < 0.0001$]. (B), After treatment with placebo: $y = 0.72x + 0.573$ g/L [SE of the slope (95% CI), 0.007 (0.158−0.185); $r^2 = 36.03\%$; $P < 0.0001$].

ment from the American Diabetes Association and the American College of Cardiology *(13)*.

Discussion

The clinical importance of measuring apo B or non-HDLC may not be confined to a more accurate prediction of risk *(8, 9)*. These analytes may also provide a better target for optimizing statin treatment. Previous studies have drawn attention to the smaller decrease in apo B relative to that of LDLC achieved with statin treatment *(8, 27)*. In agreement with such findings, patients who received atorvastatin in CARDS had a 24% decrease in apo B, whereas LDLC decreased by a mean of 41%. Clearly, the argument that the LDLC concentration is a poor indicator of the statin-induced absolute decrease in LDL particle concentration is true. From our findings for the diabetic patients assigned to

Table 2. apo B concentrations obtained from the linear-regression equations in Figs. 1 and 2 at the various LDLC and non-HDLC targets for statin therapy proposed in the ATPIII and various European recommendations for patients receiving atorvastatin, compared with apo B concentrations obtained for a similar regression analysis of patients receiving placebo with spontaneously occurring concentrations.

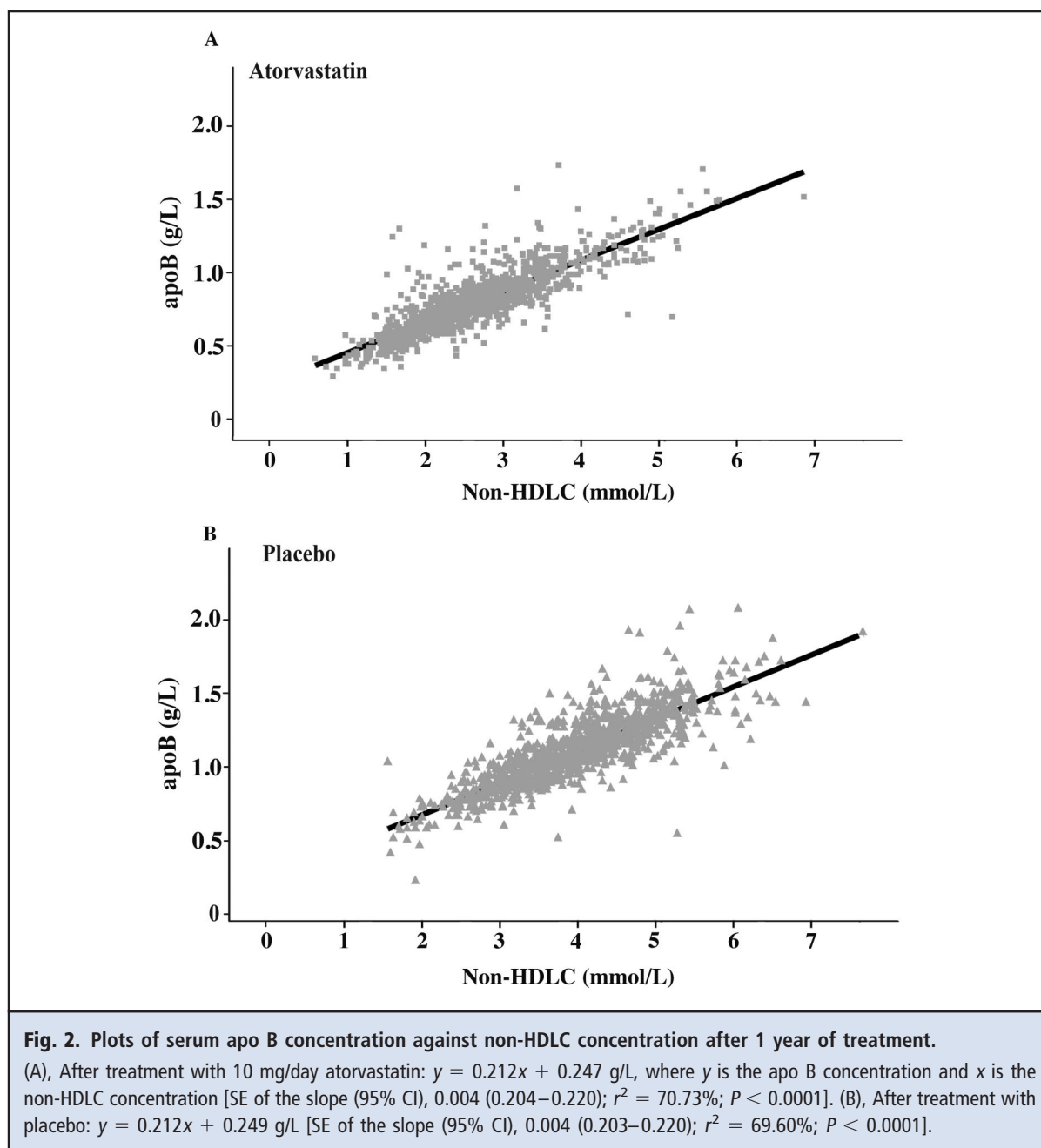| Critical target value	apo B on placebo, g/L	apo B on atorvastatin, g/L
LDLC		
3.37 mmol/L (130 mg/dL)[a]	1.151	1.112
3.00 mmol/L (116 mg/dL)[b]	1.088	1.036
2.59 mmol/L (100 mg/dL)[a]	1.018	0.949
2.00 mmol/L (77 mg/dL)[b]	0.917	0.826
1.81 mmol/L (70 mg/dL)[a]	0.884	0.787
Non-HDLC		
2.59 mmol/L (100 mg/dL)[a]	0.797	0.796
3.37 mmol/L (130 mg/dL)[a]	0.962	0.960
4.14 mmol/L (160 mg/dL)[a]	1.126	1.125

[a] ATPIII recommendations.
[b] European guidelines.

placebo, however, apo B concentrations at spontaneously occurring low LDLC concentrations appear to be typically higher than apo B concentrations achieved with atorvastatin therapy. This result means that cholesterol-depleted LDL particles are not retained in the circulation as a consequence of statin treatment, at least in the case of atorvastatin. This observation is important because apo B is the moiety that participates in the atherogenic process (4); thus, the logical aim of therapy would be to remove the entire LDL particle from the circulation. Our study also reveals that treatment targets for statin therapy will be misleading if they are based on spontaneously occurring LDLC concentrations reported in epidemiologic studies, rather than on LDLC concentrations induced by statin therapy.

Our findings raise some fundamental questions about changes in LDL composition and physical characteristics on statin therapy. Statin trials consistently show that although the apo B concentration in serum decreases in parallel with the LDLC concentration, it decreases proportionally less (8, 9). Because LDL particles each contain only a single apo B molecule, the apo B concentration is generally assumed to represent the LDL particle concentration (9). The size of the LDL particle is directly related to its lipid content, and its

density is inversely related to the amount of the lipid component. Thus, LDL particles in patients undergoing statin treatment would be predicted to be larger and less dense on average. Consistent with this expectation, we (in CARDS) and others have reported a decrease in the concentration of smaller, more dense LDL to be associated with a tendency for the average LDL particle size to increase (28–33). Of note is that the reports that described the greatest decreases in smaller, dense LDL particles were for studies with the more potent statins, atorvastatin and rosuvastatin. These reports therefore indicate that the explanation for the observed effect of atorvastatin in the present study of lowering LDLC more than apo B is unlikely to be due to an accumulation of small, dense, apo B–rich, and cholesterol-depleted LDL particles in the circulation. Rather, the effect is more likely to be due to a statin-induced increase in the catabolism of larger, cholesterol-rich, and relatively apo B–deficient LDL particles, such as intermediate-density lipoproteins and more buoyant LDL particles. This explanation would be expected from what is known of the mechanism by which statins lower LDLC, which involves an increase in hepatic LDL catabolism due to the up-regulation of LDL receptors (3, 28, 34, 35). These receptors are known to have a greater affinity for larger, cholesterol-rich LDL particles than for smaller, cholesterol-depleted ones (36). The reports of a statin-induced decrease in small, dense LDL particles are thus likely to be predominantly due to a decrease in these particles' larger LDL precursor molecules. Some of the greater decrease in the LDLC concentration relative to that of the apo B concentration may also be due to the decreased transfer of cholesteryl ester to VLDL, which occurs with statin treatment (32, 37, 38) and which would be expected to decrease the entry of cholesteryl ester into the LDL pool from VLDL. Whatever the mechanism for our findings, they emphasize the need to realize that conclusions about LDL particle concentration cannot be drawn from LDLC measurements. On the other hand, apo B concentrations were similar when patients receiving placebo or atorvastatin were standardized for their non-HDLC concentration. This observation was unlikely to be solely attributable to non-HDL lipoproteins' also including the apo B in VLDL, because only a small fraction of total apo B in serum is present in VLDL. It probably can also be because at the ATPIII cutpoints for non-HDLC, we were selecting out patients on statins who had retained higher concentrations of apo B–rich, small, dense LDL particles, which are closely related to the concentrations of VLDL (7). This finding potentially has great practical importance and supports the recent consensus statement from the American Diabetes Association and the American College of Cardiology that both apo B and non-HDLC

Fig. 2. Plots of serum apo B concentration against non-HDLC concentration after 1 year of treatment.

(A), After treatment with 10 mg/day atorvastatin: $y = 0.212x + 0.247$ g/L, where y is the apo B concentration and x is the non-HDLC concentration [SE of the slope (95% CI), 0.004 (0.204−0.220); $r^2 = 70.73\%$; $P < 0.0001$]. (B), After treatment with placebo: $y = 0.212x + 0.249$ g/L [SE of the slope (95% CI), 0.004 (0.203−0.220); $r^2 = 69.60\%$; $P < 0.0001$].

should be more widely explored as more effective targets of statin treatment than LDLC *(13)*. The scatter in the graphs of the relationships of apo B to LDLC and non-HDLC reveals that many of the patients who achieve their LDLC or non-HDLC target remain above the recommended therapeutic target for apo B. Our results are also entirely consistent with the consensus statement proposal that for physicians who aim to achieve an LDLC goal of ≤1.81 mmol/L (≤70 mg/dL) with statin treatment, equivalent targets would be 2.59

mmol/L (100 mg/dL) for non-HDLC and 0.8 g/L for serum apo B *(13)*. We do not concur, however, that the more conservative LDLC goal of ≤2.59 mmol/L (≤100 mg/dL) is equivalent to an apo B concentration of <0.9 g/L. In our study of type 2 diabetes, this concentration was equivalent to an apo B concentration >1 g/L. Even at the non-HDLC goal of 3.37 mmol/L (130 mg/dL), which was chosen to be equivalent to an LDLC of 2.59 mmol/L (100 mg/dL), the serum apo B concentration of 0.96 g/L still exceeded the 0.9-g/L concentration recom-

mended as an equivalent apo B target (13). This is not an issue of standardization of the apo B assay, which is calibrated with reference to the IFCC standards.

Thus, both apo B and non-HDLC provide more consistent goals than LDLC for assessing the LDL particle response to statin therapy. There is therefore a need for further exploration of the use of apo B and non-HDLC as targets for statin therapy, because there may be additional benefit from increasing the statin dose or changing to a more potent statin in patients whose LDL particle concentrations remain relatively high despite having achieved the current LDLC targets. There appears to be more consistency in the LDL particle number (as evidenced by apo B) with LDLC and non-HDLC at the more aggressive ATPIII LDLC goal of statin therapy.

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