Apolipoprotein B but not LDL Cholesterol Is Associated With Coronary Artery Calcification in Type 2 Diabetic Whites

Seth S. Martin,1,2 Afif N. Qasim,1 Nehal N. Mehta,1 Megan Wolfe,1 Karen Terembla,1 Stanley Schwartz,3 Nayyar Iqbal,3 Mark Schutta,3 Roshanak Bagheri,4 and Muredach P. Reilly1,3

OBJECTIVE—Evidence favors apolipoprotein B (apoB) over LDL cholesterol as a predictor of cardiovascular events, but data are lacking on coronary artery calcification (CAC), especially in type 2 diabetes, where LDL cholesterol may underestimate atherosclerotic burden. We investigated the hypothesis that apoB is a superior marker of CAC relative to LDL cholesterol.

RESEARCH DESIGN AND METHODS—We performed cross-sectional analyses of white subjects in two community-based studies: the Penn Diabetes Heart Study (N = 611 type 2 diabetic subjects, 71.4% men) and the Study of Inherited Risk of Coronary Atherosclerosis (N = 803 nondiabetic subjects, 52.8% men) using multivariate analysis of apoB and LDL cholesterol stratified by diabetes status.

RESULTS—In type 2 diabetes, apoB was associated with CAC after adjusting for age, sex, and medications [Tobit regression ratio of increased CAC for 1-SD increase in apoB: 1.36 (95% CI 1.06–1.75), P = 0.016] whereas LDL cholesterol was not [1.09 (0.85–1.41), P > 0.05]. In nondiabetic subjects, both were associated with CAC [apoB 1.65 (1.38–1.96), P < 0.001; LDL cholesterol 1.56 (1.30–1.86), P < 0.001]. In combined analysis of diabetic and nondiabetic subjects, apoB provided value in predicting CAC scores beyond LDL cholesterol, total cholesterol, the total cholesterol/HDL cholesterol and triglyceride/HDL cholesterol ratios, and marginally beyond non-HDL cholesterol.

CONCLUSIONS—Plasma apoB, but not LDL cholesterol, levels were associated with CAC scores in type 2 diabetic whites. ApoB levels may be particularly useful in assessing atherosclerotic burden and cardiovascular risk in type 2 diabetes.

Apolipoprotein B (apoB) may be more useful clinically than LDL cholesterol in coronary heart disease (CHD) because it captures greater information about atherogenic particles and is not influenced by heterogeneity of particle cholesterol content (1). Measurement of LDL cholesterol is relatively insensitive to the accumulation of small, dense LDL particles, which are believed to be highly atherogenic (1). This is reflected in the preponderance of evidence from prospective epidemiologic studies and statin trials favoring apoB over LDL cholesterol as a predictor of cardiovascular risk as well as residual risk on statin therapy (2–10).

Heterogeneity of LDL particle cholesterol content is increased in type 2 diabetes because insulin resistance drives VLDL cholesterol production, leading to depletion of LDL cholesterol via the action of cholesterol ester transfer protein (CETP) (11). CETP exchanges triglycerides for cholesterol on LDL particles, which are remodeled by lipases to produce cholesterol-poor, small, dense LDL particles (11,12). Because there is one apoB per LDL particle, regardless of density, apoB detects the presence of these atherogenic particles, in contrast to LDL cholesterol, and thus may be better suited to guide lipid-lowering therapy, particularly in insulin resistance and type 2 diabetes.

Data are lacking on the relationship of apoB to coronary artery calcification (CAC), a quantitative measure of subclinical atherosclerosis and predictor of CHD in diabetes (13) as well as in the general population (14,15). Therefore, we examined the relative association of plasma apoB and LDL cholesterol with CAC in two cross-sectional studies of individuals without known CHD, one recruited based on type 2 diabetes and the other based on family history of CHD. We hypothesized that apoB levels would be stronger predictors of CAC than LDL cholesterol levels, particularly in type 2 diabetic subjects. We also hypothesized that apoB might add incremental value to traditional cholesterol-based CHD risk parameters.

RESEARCH DESIGN AND METHODS

Study participants. Details of the Penn Diabetes Heart Study (PDHS) (16,17) and the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) (18–20) have been reported previously. In brief, both are contemporary, single-center, cross-sectional community-based studies of subjects without clinical evidence of CHD (defined as myocardial infarction, coronary revascularization, angiographic disease, or positive stress test) recruited at the University of Pennsylvania that used the same clinical research center, research staff, electron beam computed tomography scanner, and lipid laboratory. SIRCA subjects were recruited in 1995–2005 based on a family history of premature CHD. PDHS subjects were recruited in 2001–2007 based on type 2 diabetes.
Exclusion criteria included clinical CHD, elevated creatinine, and, in SIRCA, the presence of diabetes. This report focuses on unrelated, white subjects (diabetic participants n = 611, nondiabetic participants n = 803).

**Evaluated parameters.** Participants were evaluated at the General Clinical Research Center at the University of Pennsylvania Medical Center after a 12-h overnight fast. apoB and plasma lipids were measured in Penn’s Center for Disease Control–certified lipid laboratory. Standard lipid panels and apoB were measured enzymatically (Cobas Fara II; Roche Diagnostic Systems, Somerville, NJ) in lipoprotein fractions after ultracentrifugation (β-quantification technique) in PDHS (21) and in whole serum in SIRCA in a Penn’s Center for Disease Control–certified lipid laboratory (21). Analyses use LDL cholesterol calculated by the Friedewald formula; direct LDL cholesterol measurement was available for diabetic subjects and produced essentially identical results (data not shown). For apoB and C-peptide response (high sensitivity), immunoturbidimetric assays were used (16,19). Laboratory test results were generated by personnel blinded to the clinical characteristics and CAC scores of research subjects. Clinical parameters, including blood pressure and waist circumference, were assessed as previously reported (16,18). Framingham risk scores, using calculated LDL cholesterol (similar results using total cholesterol), were determined as described by Wilson et al. (22). Subjects were classified as having the metabolic syndrome using the revised National Cholesterol Education Panel definition (glucose cut point 100 mg/dl) (23).

Global CAC scores were quantified as described (18) according to the method of Agatston et al. (24) by electron beam tomography.

**Statistical analysis.** Data are reported as median (interquartile range [IQR]) or mean ± SD for continuous variables and as proportions for categorical variables. The crude association of apoB and LDL cholesterol with lipid, metabolic, and inflammatory parameters was examined by Spearman correlation. Multiple linear regression analysis of CAC scores was performed using Tobit conditional regression of natural log (CAC + 1). Tobit conditional regression is particularly suited to the unusual distribution of CAC data (many zero scores but also a marked right skew) (18,25). It combines two regression approaches: first, a logistic regression of the presence of CAC (any CAC present versus CAC zero score) and second, a linear regression of (log-transformed CAC) when CAC is present. This provides a single estimate for the relationship of risk factors with CAC data. We present Tobit ratios for CAC score increment for a 1-SD increase in a lipid parameter, which allows a similarly scaled comparison of different lipid parameters. A Tobit ratio of 1.30 means that there is a 30% increase in the CAC score for every SD increase in a lipid parameter. We also performed secondary logistic regression analysis of the presence of any CAC.

Our modeling is based on the assumption that current lipoprotein measures reflect prior levels and exposures that contributed to atherosclerosis over time. The association of apoB, LDL cholesterol, and non-HDL cholesterol levels with CAC was assessed in incremental models with increasing numbers of confounding risk factors. Model 1 was adjusted for age, sex, and medication; model 2 was additionally adjusted for atherosclerotic risk factors including hypertension, tobacco use, alcohol use, exercise, family history of premature CHD, C-reactive protein, and metabolic syndrome; whereas for apoB, model 3 was further adjusted for total cholesterol. Interaction of apoB and LDL cholesterol with type 2 diabetes was tested by likelihood ratio testing, and stratified results are presented when appropriate. Finally, we applied likelihood ratio testing in nested models to assess the incremental value of apoB over cholesterol parameters and clinical risk assessments (Framingham risk score, metabolic syndrome), and vice versa, in predicting CAC scores. Statistical analyses were performed using Stata 10.0 software (Stata, College Station, TX).

**RESULTS**

**Characteristics of study samples.** Table 1 summarizes study sample characteristics stratified by type 2 diabetes status. Diabetic subjects were older, predominantly male, more obese, and had lower total and LDL cholesterol as well as apoB levels (P < 0.001 for all), likely reflecting greater use of statin therapy. Fifteen percent of those with diabetes were on insulin, and median A1C was 6.8%. As expected, National Cholesterol Education Panel–defined metabolic syndrome was present in over 75% of type 2 diabetic and ~25% of nondiabetic patients. The correlation of apoB with LDL cholesterol was similar in diabetic (r² = 0.67) and nondiabetic (r² = 0.64) subjects. Spearman correlations revealed associations of apoB and LDL cho-

| TABLE 1 | Characteristics of the study sample |
|---------|-------------------------------------|
|          | Type 2 diabetic subjects | Nondiabetic subjects |
| n        | 611                      | 803                     |
| Age (years) | 60 (54–68)              | 48 (42–54)              |
| Male (%)  | 71.4                     | 52.8                    |
| Total cholesterol (mg/dl) | 174 (152–198)          | 205 (177–228)           |
| HDL cholesterol (mg/dl)  | 46 (37–53)              | 48 (39–59)              |
| Triglycerides (mg/dl)    | 134 (92–197)            | 117 (87–159)            |
| LDL cholesterol (mg/dl)  | 97 (79–119)             | 126 (103–148)           |
| apoB (mg/dl)             | 82 (71–94)              | 98 (84–114)             |
| Medications             |                        |                        |
| Statin (%)              | 57.4                    | 13.9                    |
| Niacin (%)              | 5.6                     | 3.0                     |
| Fibrate (%)             | 10.0                    | 11.1                    |
| Insulin (%)             | 14.9                    | N/A                     |
| Metformin (%)           | 63.8                    | N/A                     |
| Thiazolidinediones (%)  | 27.3                    | N/A                     |
| Sulfonylureas (%)       | 40.3                    | N/A                     |
| Ten-year Framingham risk (%) | 13 (8–20)              | 5 (3–8)                  |
| Current smoking (%)     | 8.4                     | 11.3                    |
| Alcohol use (%)         | 58.4                    | 67.8                    |
| Blood pressure (mmHg)   |                        |                        |
| Systolic                | 131 (122–140)           | 126 (117–136)           |
| Diastolic               | 75 (71–81)              | 77 (72–84)              |
| BMI (kg/m²)             | 32 (28–36)              | 27 (24–30)              |
| Waist circumference (cm) | 107 (98–117)           | 89 (81–99)              |
| Metabolic syndrome (%)  | 76.6                    | 25.8                    |
| C-reactive protein (mg/dl) | 1.6 (0.8–3.4)       | 1.2 (0.5–2.6)           |

CAC

Mean score (± SD) 424 ± 795 87 ± 266
Median (IQR) 89 (1–456) 3 (0–45)
>0 (%) 75.3 68.9
≥100 (%) 49.1 16.4
≥400 (%) 26.8 5.4

Data are median (IQR) or percent, unless otherwise noted.

**Plasma levels of apoB, but not of LDL cholesterol, are associated with CAC in diabetic participants.** In type 2 diabetic whites (Table 3, left columns), apoB [Tobit ratio for 1-SD increase, 1.36 (95% CI 1.06–1.75), P = 0.016], but not LDL cholesterol, was associated with CAC

| TABLE 2 | Spearman correlations of lipid, metabolic, and inflammatory variables with plasma apoB and LDL cholesterol |
|---------|---------------------------------------------------------------|
|          | Type 2 diabetic subjects (n = 611) | Nondiabetic subjects (n = 803) |
|          | ApoB | LDL cholesterol | ApoB | LDL cholesterol |
| Total cholesterol | 0.78‡ | 0.90‡ | 0.77‡ | 0.90‡ |
| HDL cholesterol | -0.21‡ | 0.02 | -0.21‡ | -0.03 |
| Triglycerides | 0.47‡ | 0.15‡ | 0.51‡ | 0.18‡ |
| Glucose | 0.20‡ | 0.07 | 0.12‡ | 0.02 |
| Waist circumference | 0.08* | -0.004 | 0.25‡ | 0.13‡ |
| Framingham risk | 0.43‡ | 0.41‡ | 0.50‡ | 0.38‡ |
| Blood pressure |                      |                |              |
| Systolic | 0.05 | -0.01 | 0.20‡ | 0.14‡ |
| Diastolic | 0.14‡ | 0.05 | 0.20‡ | 0.12‡ |
| C-reactive protein | 0.17‡ | 0.05 | 0.25‡ | 0.12‡ |

*P < 0.05, †P < 0.01, ‡P < 0.001.
not LDL cholesterol [1.09 (0.85–1.41)], was associated with CAC after adjusting for age, sex, and lipid-lowering and diabetes medications. In nondiabetic patients (Table 3, right columns), both apoB [1.65 (1.38–1.96), \( P < 0.001 \)] and LDL cholesterol [1.56 (1.30–1.86), \( P < 0.001 \)] were associated with CAC in this simple model. After further adjusting for multiple cardiovascular risk factors, this pattern of CAC association persisted (Table 3). Even after adjusting for total cholesterol, apoB continued to have a strong association with CAC in diabetic subjects [1.83 (1.17–2.85)], whereas in nondiabetic subjects, this was attenuated [1.22 (0.90–1.65)]. In combined analysis of diabetic and nondiabetic subjects, interaction analysis suggested a consistent CAC association for apoB (diabetes interaction \( P = 0.25 \)), but a difference by diabetes status in the relationship of LDL cholesterol with CAC (interaction \( P = 0.02 \)). Results of logistic regression of the presence of CAC were similar to that for Tobit modeling (see Appendix Table 1, available online at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1794/DC1). To explore the effect of greater statin use in the diabetic versus nondiabetic samples, we performed a secondary analysis excluding statin users. These analyses yielded analogous findings to the full sample for the pattern of apoB, LDL cholesterol, and other lipid relationships with CAC (Appendix Table 2 in the online appendix).

In diabetic participants, plasma levels of non-HDL cholesterol had stronger CAC association than LDL cholesterol but less than that for apoB. In contrast, non-HDL cholesterol had almost identical CAC association as apoB in nondiabetic whites (Table 3; Appendix Tables 1 and 2 in the online appendix).

**Incremental value of apoB levels over cholesterol-based risk parameters.** We combined data across diabetic and nondiabetic subjects and assessed the incremental value of apoB over cholesterol parameters (Table 4, *top rows*). ApoB added value in predicting CAC scores when added to LDL cholesterol and total cholesterol. In fact, it also added value to the total cholesterol/HDL cholesterol and triglyceride/HDL cholesterol ratios and marginally to non-HDL cholesterol. In contrast, adding LDL cholesterol, total cholesterol, and non-HDL cholesterol to apoB failed to provide additional value (Table 4, *bottom rows*). As expected, because they contain HDL data, the total cholesterol/HDL cholesterol and triglyceride/HDL cholesterol ratios added value to apoB in predicting CAC score. Notably, apoB provided incremental value in predicting CAC beyond Framingham risk scores, suggesting utility beyond current approaches in clinical practice. In secondary analyses, stratified by diabetes status, apoB also added value to LDL cholesterol in those with and without type 2 diabetes (Appendix Table 3). ApoB's additive value to non-HDL cholesterol and HDL cholesterol containing parameters was attenuated, however, likely because of reduced power in the smaller strata.

### TABLE 3
Association of plasma levels of apoB and cholesterol parameters with CAC

| Variables adjusted for | Type 2 diabetic subjects \((n = 611)\) |
|------------------------|----------------------------------|
|                        | *Tobit ratio (95% CI) | Nondiabetic subjects \((n = 803)\) |
|                        | *Tobit ratio (95% CI) | |
| ApoB                   | 1.36 (1.06–1.75) | 1.65 (1.38–1.96) |
| Age, sex, medications  | 1.37 (1.05–1.79) | 1.50 (1.25–1.80) |
| LDL cholesterol        | 1.09 (0.85–1.41) | 1.56 (1.30–1.86) |
| Age, sex, medications  | 1.13 (0.87–1.47) | 1.51 (1.27–1.81) |
| Non-HDL cholesterol    | 1.30 (1.01–1.68) | 1.68 (1.41–2.00) |
| Age, sex, medications  | 1.28 (0.99–1.67) | 1.54 (1.29–1.85) |
| Non-HDL cholesterol    | 1.14 (0.87–1.54) | 1.35 (1.09–1.67) |
| Age, sex, medications  | 1.18 (0.91–1.51) | 1.35 (1.08–1.67) |
| Non-HDL cholesterol    | 1.20 (0.91–1.54) | 1.40 (1.14–1.71) |

Results of Tobit conditional regression are presented as the ratio of increase in CAC score for 1-SD increase in apoB (17.84 mg/dl in diabetic subjects; 22.83 mg/dl in nondiabetic subjects), LDL cholesterol (31.63 mg/dl in diabetic subjects; 35.08 mg/dl in nondiabetic subjects), or non-HDL cholesterol (36.91 mg/dl in diabetic subjects; 38.79 mg/dl in nondiabetic subjects). *Tobit ratio of 1.36 means that for every 17.84 mg/dl increase in apoB, there is a 36% increase in the CAC score. Medications included statins, niacin, fibrates, insulin, metformin, thiazolidinediones, sulfonylureas, and hormone replacement therapy. Risk factors included hypertension, tobacco use, alcohol use, exercise, family history of premature cardiovascular disease, C-reactive protein, and metabolic syndrome.

### TABLE 4
Relative value of apoB and cholesterol parameters in predicting CAC scores in diabetic and nondiabetic subjects

| Cholesterol parameter(s) added to apoB | All subjects \((n = 1,414)\) |
|----------------------------------------|----------------------|
|                                       | \( \chi^2 \) | \( P \) value |
| apoB added to                           |                     |
| LDL cholesterol                        | 15.26               | <0.001         |
| Total cholesterol                      | 16.65               | <0.001         |
| Non-HDL cholesterol                    | 3.2                 | 0.07           |
| HDL cholesterol                        | 24.37               | <0.001         |
| Triglyceride/HDL cholesterol ratio     | 17.31               | <0.001         |
| Total cholesterol/HDL cholesterol ratio| 4.32                | 0.04           |
| Framingham risk score and metabolic syndrome | 16.09 | <0.001 |

Likelihood ratio testing was applied in nested Tobit models to assess the incremental value of apoB over cholesterol parameters, and vice versa, in predicting CAC scores. All models included age, sex, medications, and diabetes status.
DISCUSSION

We report that apoB, but not LDL cholesterol, was associated with CAC scores in type 2 diabetic whites. This was true despite relatively high correlations of these two lipid parameters in diabetic subjects. In contrast, both apoB and LDL cholesterol were equally associated with CAC in nondiabetic patients. Although non-HDL cholesterol was superior to LDL cholesterol, we found that apoB added incremental value to total cholesterol and LDL cholesterol and even tended to add to non-HDL cholesterol in CAC prediction, whereas the reverse was not true. Overall, these findings support the concept that apoB levels may be stronger predictors of atherosclerotic burden than LDL cholesterol and other cholesterol parameters in type 2 diabetes.

Robust evidence from large primary and secondary prevention clinical trials established the standard practice of LDL cholesterol lowering for CHD prevention (26). Contemporary data have refined our interventions toward more aggressive therapeutic targets (27), yet the majority of CHD events are not prevented. One potential means of improving outcomes is through more precise estimation of atherogenic lipoprotein parameters, beyond cholesterol content, that more fully capture CHD risk.

ApoB, a measure of LDL particle number (LDL-P), as well as total atherogenic particle number, may represent such a parameter. In most (2–10), but not all (28–30), prior reports, including several large, prospective epidemiological studies and clinical trials, apoB surpassed LDL cholesterol and other cholesterol parameters, as a predictor of new and recurrent CHD events and marker of residual risk on therapy. Concordantly, apoB is the top performer in our report and recurrent CHD events and marker of residual risk. Among the laboratory methods that currently exist for determination of LDL-P, apoB is the most mature and cost-effective. It is broadly equivalent to LDL-P because each LDL particle, independent of density, contains exactly one apoB and the vast majority (≥90%) of apoB is carried on LDL particles (32). In this way, apoB is not affected by heterogeneity of particle cholesterol content. Such heterogeneity is greater in type 2 diabetes because insulin resistance drives VLDL cholesterol production that depletes LDL particles of their cholesterol content via CETP, producing cholesterol-poor small, dense LDL particles (11). Remarkably, the remainder of apoB is carried on chylomicrons, VLDL cholesterol, intermediate-density lipoprotein, and lipoprotein(a) and thus also captures information on residual non-LDL atherogenic particles. ApoB measurement is standardized (37) and automated, yielding cost, time, and precision advantages over other modalities. It is available in most large commercial laboratories and does not require a fasting state. Thus, apoB has several measurement-related advantages as a marker of lipid risk.

Nuclear magnetic resonance (NMR) is an alternative means of measuring LDL-P; however, its clinical utility is currently limited because it is expensive and not widely available across laboratories. Nevertheless, reports on its predictive power in CHD are revealing. NMR-measured LDL-P improved cardiovascular risk estimation over LDL cholesterol in several cross-sectional (38) and prospective studies (39–43). Cross-sectional studies of healthy individuals showed that LDL-P was associated with CAC in postmenopausal women (44) and carotid intima-media thickness in the 5,538-person Multi-Ethnic Study of Atherosclerosis study (38). Prospectively, LDL-P predicted incident CHD in healthy (39,41,43) and at-risk (42) populations, as well as progression of CHD (40). In a nested case-control analysis of 2,888 subjects from the European Prospective Investigation Into Cancer and Nutrition-Norfolk, LDL-P outperformed LDL cholesterol as a predictor of future coronary artery disease beyond the Framingham risk score, but not triglycerides and HDL cholesterol (43). Although NMR has the capacity to estimate LDL particle size as well as particle number (43), there is limited evidence that NMR-estimated lipid data add value beyond the more simple measurement of apoB (33,41,42).

Patients with type 2 diabetes tend to have increased circulating LDL particles but normal concentrations of LDL cholesterol because their particles have low cholesterol content (45). Despite elevated triglycerides and low HDL cholesterol, this normal LDL cholesterol has led to underappreciation of the risk associated with dyslipidemia in diabetes. Indeed, in type 2 diabetic subjects, apoB and non-HDL cholesterol were favored over LDL cholesterol as predictors of CHD risk in the Health Professional’s Follow-Up Study (31). Our data also shows apoB and non-HDL cholesterol capture information beyond LDL cholesterol in type 2 diabetes. We go further, in agreement with the recent Casale Monferrato Study (46) and Collaborative Atorvastatin Diabetes Study (47), in suggesting utility of apoB measurement over cholesterol parameters, including non-HDL cholesterol. Casale Monferrato looked at 11-year CHD mortality in 1,565 Mediterranean subjects with type 2 diabetes and found apoB predicted outcome independent of non-HDL cholesterol. CARDS followed

ApO AND CORONARY CALCIFICATION IN TYPE 2 DIABETES

[1890]
2,627 type 2 diabetic participants for 108 primary CHD end points over 3.9 years. ApoB carried a very similar hazard ratio to non-HDL cholesterol [adjusted hazard ratio (95% CI) for 1-SD increment: 1.20 (1.04–1.38) vs. 1.17 (1.02–1.34), respectively], but apoB was the stronger predictor ($\chi^2 = 6.61; P = 0.01$ vs. $\chi^2 = 4.71; P = 0.03$). In receiver operating characteristic analysis, the area under the curve for apoB versus non-HDL cholesterol was significantly greater ($P = 0.01$). Overall, our data support others' in suggesting that apoB is likely to be an enhanced measure of subclinical coronary atherosclerosis and lipid-associated CHD risk beyond traditional lipid risk factors in type 2 diabetes.

Our study has several limitations. Analyses were cross-sectional; thus, causal and longitudinal relationships were not addressed. ApoB's stronger association with CAC might reflect less variability in its measurement, especially over time, relative to LDL cholesterol. However, we cannot differentiate this possibility from a true stronger apoB association with CAC in our cross-sectional study. We also did not examine clinical outcomes, although our data are consistent with large clinical outcomes studies. Moreover, given lipid (48) and CAC (49) variability by race, our findings cannot be generalized beyond whites. In addition, CAC is an estimate (14,50) and not a direct measure of coronary atherosclerosis; thus, it may fail to detect some coronary atherosclerotic plaques. Despite this limitation, CAC scores are clinically relevant because they are strong, independent predictors of CHD (14,15), including in diabetic subjects (13). In our study, there was also extensive and differential statin use between diabetic and nondiabetic participants. Although this could confound the results, it represents real-world practice. In fact, we found that apoB predicted CAC even after controlling for differences in statin use and in subgroup analysis of nonstatin users.

Ours is the first study to show that plasma apoB, but not LDL cholesterol, levels are associated with CAC beyond traditional risk factors in type 2 diabetic whites. LDL cholesterol and cholesterol-related parameters did not add value to apoB. These results for subclinical coronary atherosclerosis agree with clinical outcomes data supporting apoB as a predictor of cardiovascular events. Our findings are broadly consistent with a recent joint consensus statement from the American Diabetes Association and American College of Cardiology that recommends incorporating apoB in managing patients with cardiometabolic risk (33). We advance previous apoB literature by addressing its relationship to subclinical coronary atherosclerosis in type 2 diabetic patients free of clinical CHD and we provide data that apoB may warrant greater use in risk assessment beyond LDL cholesterol in these asymptomatic individuals at higher cardiometabolic risk.

REFERENCES

1. Mudl JO, Borlaug BA, Johnston PV, Kral BG, Roff R, Blumenthal RS, Kwitterovich PJ Jr. Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. J Am Coll Cardiol 2007;50:1735–1741

2. Lamarache B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. Circulation 1997;95:69–75

3. St-Pierre AC, Cantin B, Dagenais GR, Maurriege P, Bernard PM, Despres JP, Lamarache B. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol 2005;25:553–559

4. Pischon T, Girman CJ, Sacks FM, Rifai N, Stamper MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. Circulation 2005;112:3575–3583

5. Barter PJ, Ballantyne CM, Carmena R, Castro Cabezas M, Chapman MJ, Couture P, de Graaf J, Durrington PN, Faergeman O, Frohlich J, Furbager CD, Gagne C, Haffner SM, Humphries SE, Jungner I, Krauss RM, Kwitterovich P, Marcovina S, Packard CJ, Pearson TA, Reddy KS, Rosenson R, Sarrazaud P, Sniderman AD, Stalenhoef AF, Stein E, Talman PJ, Tonkin AM, Waldgus S, Williams KM. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the 30-person/10-country panel. J Intern Med 2006;299:247–258

6. Waldgus S, Jungner I, Holme I, Aastveit AH, Kolar W, Steinr E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 2001;358:2026–2033

7. Moos AJ, Goldstein RE, Marder VJ, Sparks CE, Oakes D, Greenberg H, Weiss HJ, Zareba W, Brown MW, Liang CS, Liddle WC, Gillespie JA, Van Voorhees L, Krone RJ, Bodenheimer MM, Hochman J, Dwyer EM, Jr, Arora R, Marcus FI, Watle LF, Case RB. Thrombogenic factors and recurrent coronary events. Circulation 1999;99:2517–2522

8. Benn M, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Improved prediction of ischemic cardiovascular disease in the general population using apolipoprotein B: the Copenhagen City Heart Study. Arterioscler Thromb Vasc Biol 2007;27:661–670.

9. van Lennep JE, Mertens J, Van Lennep HW, Zwinderman AH, Erkels DW, van der Wall EE. Apolipoprotein concentrations during treatment and recurrent coronary artery disease events. Arterioscler Thromb Vasc Biol 2000;20:2408–2413

10. Goto AM Jr, Whitney E, Stein EA, Shapiro DR, Clearfield M, Weis S, Joo JY, Langendorfer A, Beere PA, Watson DJ, Downs JF, de Canis JS. Relation between baseline and on-treatment cholesterol pathogen and acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAP/TEXCAP). Circulation 2000;101:477–484

11. Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyper-apob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. Ann Intern Med 2001;135:447–456

12. Brown RJ, Rader DJ. Lipases as modulators of atherosclerosis in murine models. Curr Drug Targets 2007;8:1307–1319

13. Ellefson RS, Godalski IF, Feher MD, Rubens MB, Roughton M, Nugra F, Humphries SE, Richardson W, Flather MD. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. Eur Heart J 2008;29:2244–2251

14. Rumberger JA, Brundage BH, Rader DJ, Kondos G. Electron beam computed tomographic coronary calcium scanning: a review and guidelines for use in asymptomatic persons. Mayo Clin Proc 1999;74:243–252

15. Fletcher MJ, Tice JA, Pignone M, Brower WS. Using the coronary artery calcium score to predict coronary heart disease events: a systematic review and meta-analysis. Arch Intern Med 2004;164:1285–1292

16. Reilly MP, Igbal N, Schutta M, Wolfe ML, Scally M, Localio AR, Rader DJ, Kimmel SE. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. J Clin Endocrinol Metab 2004;89:3872–3878

17. Wolfe ML, Igbal N, Gefter W, Mohler ER 3rd, Rader DJ, Reilly MP. Coronary artery calcification at electron beam computed tomography is increased in asymptomatic type 2 diabetics independent of traditional risk factors. J Cardiovasc Risk 2002;9:360–367

18. Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular risk factors: impact of the analytic approach. Atherosclerosis 2004;173:68–78

19. Reilly MP, Lehrke M, Wolfe ML, Rolhati A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 2005;111:932–939

20. Qasim A, Mehta NN, Tadesse MG, Wolfe ML, Rhodes T, Girman C, Reilly
MP. Adipokines, insulin resistance, and coronary artery calcification. J Am Coll Cardiol 2008;52:231–236

21. Hirany S, Li D, Jialal I. A more valid measurement of low-density lipoprotein cholesterol in diabetic patients. Am J Med 1997;102:48–53

22. Wilson PW, D’Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837–1847

23. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Sperut JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735–2752

24. Agatston AS, Janowitz WR, Hildner FJ, Zesmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1999;15:827–832

25. Tobin J. Estimation of relationships for limited dependent variables. Econometrica 1958;26:24–36

26. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. JAMA 2001;285:2486–2497

27. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004;110:227–239

28. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. JAMA 2005;294:326–333

29. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keys MJ, Pencina MJ, Schoonmaker C, Wilson PW, D’Agostino RB, Vasan RS. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007;298:776–785

30. van der Steeg WA, Boekholdt SM, Stein EA, El-Harchaoui K, Stroes ES, Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, Ridker PM. Non-HDL cholesterol, apolipoprotein B, and coronary events among men with type 2 diabetes. Diabetes Care 2004;27:1991–1997

31. van der Steeg WA, Boekholdt SM, Stein EA, EL-Harchaoui K, Stroes ES, Sandhu MS, Wareham NJ, Jukema JW, Luben R, Zwierdeman AH, Kastelein JJ, Khaw KT. Role of the apolipoprotein B-apolipoprotein A-I ratio in cardiovascular risk assessment: a case-control analysis in EPIC-Norfolk. Ann Intern Med 2007;146:640–648

32. Snieder AD, Marcovina SM. Apolipoprotein A-I and B. Clin Lab Med 2006;26:731–750

33. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, Witztum JL. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. J Am Coll Cardiol 2006;51:1512–1524

34. McQueen MJ, Hawken S, Wang X, Oumpu S, Snieder A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, Kazuki K, Yasus S. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. Lancet 2008;372:224–233

35. Rader DJ. High-density lipoproteins and atherosclerosis. Am J Cardiol 2002;90:62–70

36. Stamper MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276:882–888

37. Marcovina SM, Albers JJ, Kennedy H, Mei JV, Henderson LO, Hannon WH. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of International Reference Material. Clin Chem 1994;40:586–592

38. Mora S, Szkl M, Otvos JD, Greenland P, Psaty BM, Goff DC Jr, O’Leary DH, Saud MF, Tsai MY, Sharrett AR. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2007;192:211–217

39. Kuller L, Arnold A, Tracy R, Otvos J, Burke G, Psaty B, Siscovich D, Freedman DS, Kronmal R. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the cardiovascular health study. Arterioscler Thromb Vasc Biol 2002;22:1175–1180

40. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002;90:89–94

41. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002;106:1930–1937

42. Otvos JD, Collins D, Freedman DS, Shalavoula I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ. Lip- density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably altered by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006;113:1556–1563

43. El Harchaoui K, van der Steeg WA, Stroes ES, Kuivenhoven JA, Otvos JD, Wareham NJ, Huttten BA, Kastelein JJ, Khaw KT, Boekholdt SM. Value of low-density lipoprotein particle number and size as predictors of coronary artery disease in apparently healthy men and women: the EPIC-Norfolk Prospective Population Study. J Am Coll Cardiol 2007;49:547–553

44. Mackey RH, Kuller LH, Sutton-Tyrrell K, Evans RW, Holubkov R, Matthews KA. Lipoprotein subclasses and coronary artery calcium in postmenopausal women from the healthy women study. Am J Cardiol 2002;90:711–716

45. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. Am J Cardiol 2002;90:228–29

46. Bruno G, Merletti F, Biggeri A, Bargero G, Prina-Cerai S, Pagano G, Cavallo-Perin P. Effect of age on the association of non-high-density lipoprotein cholesterol and apolipoprotein B with cardiovascular mortality in a Mediterranean population with type 2 diabetes: the Casale Monferrato study. Diabetologia 2006;49:937–944

47. Charlton-Menys V, Betteridge DJ, Colhoun H, Fuller J, France M, Hitman GA, Livingston SJ, Neil HA, Newcom CB, Szarek M, DeMicco DA, Durrington PN. Apolipoproteins, cardiovascular risk, and statin response in type 2 diabetes: the Collaborative Atorvastatin Diabetes Study (CARDS). Diabetologia 2009;52:218–225

48. Kuller LH. Ethnic differences in atherosclerosis, cardiovascular disease and lipid metabolism. Curr Opin Lipidol 2004;15:109–113

49. Nasir K, Shaw LJ, Liu ST, Weinstein SR, Mosler TR, Flores PR, Flores FR, Byrd BS, Yee C, Alpert JS, D’Agostino RB. MRI to quantify coronary artery calcium: ultrafast electron beam CT and histomorphometric correlation. Radiology 1994;192:619–623