## Materials and Methods

### Study participants

Subjects included in this investigation were recruited from the Penn Diabetes Heart Study (PDHS), a cohort which has been described in detail previously [1, 2]. In brief, PDHS is a community-based, cross-sectional cohort for the study of novel risk factors for coronary atherosclerosis in patients with type 2 diabetes but without clinical cardiovascular disease. The study recruited 2032 subjects with type 2 diabetes between 2001 and 2011 from primary care and endocrinology outpatient clinics affiliated with the Hospital of the University of Pennsylvania and the Philadelphia VA Medical Center. Subjects were eligible if they were between 35 and 75 years, were previously diagnosed with type 2 diabetes (defined as fasting blood glucose ≥ 126 mg/dl, 2 hour postprandial glucose ≥ 200 mg/dl, or use of oral hypoglycemic agents/insulin in a subject greater than age 40 years) and negative pregnancy test (if female). Exclusion criteria were the presence of clinical coronary artery disease (defined as myocardial infarction, coronary revascularization, angiographic coronary disease or positive stress test), insulin use prior to age 35, a serum creatinine >2.5 mg/dl and a weight >300 lb (136.4 kg). Of the 2032 subjects originally recruited, plasma samples were available from 1422 subjects for ApoC-III measurement at the time this investigation was initiated, and these subjects were included here. University of Pennsylvania IRB approval was obtained for this study and informed consent was obtained from all study subjects. In this study, metabolic syndrome was defined according to the National Cholesterol Education Program ATP III Guidelines. Briefly, metabolic syndrome was defined as the presence of any 3 of the following 5 conditions in a participant: 1) obesity (waist circumference >40 inches for males or >35 inches for females), 2) hyperglycemia (fasting glucose ≥ 100 mg/dl or treatment with hypoglycemic agents/insulin), 3) dyslipidemia (fasting TG ≥ 150 mg/dl or treatment for dyslipidemia), 4) hypoalphalipoproteinemia (HDL-C < 40 mg/dl for males or < 50 mg/dl for females or treatment for low HDL-C), 5) hypertension (systolic BP > 130 mm Hg, diastolic BP > 85 mm Hg or treatment for hypertension). The criteria used for defining the metabolic syndrome was thus not mutually exclusive for the concomitant diagnosis of type 2 diabetes.

### Measured Traits

Eligible subjects were evaluated at Penn's Clinical and Translational Research Center (CTRC) after a 12-h overnight fast. All individuals underwent a detailed questionnaire for medical information and anthropometric measurements. All lipid and apolipoprotein quantitation was performed by enzymatic assays in a Center for Disease Control-certified lipid laboratory at the University of Pennsylvania [2]. Total cholesterol and TG were measured in plasma, HDL cholesterol (HDL-C) was measured after precipitation, and VLDL cholesterol (VLDL-C) and LDL cholesterol (LDL-C) after ultracentrifugation (β-quantification) (Hitachi 912, Roche Diagnostic Systems Inc., NJ, USA). Plasma apolipoprotein (apo) levels for apoB, apoA-I, apoA-II, apoE, and apoC-III were measured using Roche reagents on the Hitachi 912. C-Reactive Protein (CRP) levels were measured as described previously [3]. Plasma levels of adiponectin, leptin (Linco Research, St. Charles, MO, USA), and insulin (Linco, St Charles MO), as well as IL-6 (R+D Systems, Minneapolis) were measured by enzyme-linked immunosorbent assays

(ELISAs). Participants were classified as having the metabolic syndrome using the revised National Cholesterol Education Program (NCEP) criteria. Global CAC scores were determined using the method of Agatston *et al*. from 40 continuous 3 mm-thick computed tomograms collected on an EBT scanner using customized software (Imatron, San Francisco, CA) [1, 2]. Laboratory test results and CT data were generated by personnel blinded to the clinical characteristics of study participants.

**Statistical Analysis**

Statistical analyses were performed using Stata version 12.0 (Stata Corp LP, College Station, Texas). Descriptive characteristics were performed for key clinical risk factors through the use of mean, median, and interquartile ranges for continuous variables and percentages for categorical variables. The unadjusted correlation of plasma ApoC-III concentration with quantitative lipid, metabolic, and inflammatory biomarker measurements was examined using Spearman's correlation analysis. Correlations of ApoC-III with TG were performed using log-transformed TG levels because baseline TG measurements were not normally distributed. A linear regression analysis was also performed for these variables after adjusting for age, gender, race, BMI, history of smoking, alcohol use, estimated glomerular filtration rate (eGFR) and medications that affect glucose and lipoprotein levels.

The primary analysis of the relationship between apoC-III and CAC was performed using tobit conditional regression of ln (CAC+1) as described previously; this approach is well suited to the presence of many zero scores but substantial rightward skew of the CAC phenotype [2, 3]. Similar analysis implemented tobit regression after assignment of participants to ascending quartiles of apoC-III levels. A final analysis of logistic regression of CAC dichotomized as present (score > 0) vs. absent (score = 0) was also performed. Multivariate models included 1) age, gender, race; 2) additionally BMI, history of smoking, alcohol use, eGFR, ln CRP statin, fibrates, nicain, ezetimibe, thiazolidinediones (TZD), metformin, sulfonylurea, insulin, exenatide and sitagliptan, and 3) further addition of TG. P values of less than 0.05 were considered to be statistically significant.

## References


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