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Association of Apolipoprotein B, LDL-C and vascular stiffness in Adolescents with Type 1 Diabetes

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Abstract

Objective—LDL cholesterol (LDL-C) is the current lipid standard for cardiovascular disease (CVD) risk assessment in type 1 diabetes. Apolipoprotein B (apoB) may be helpful to further stratify CVD-risk. We explored the association between apoB and pulse wave velocity (PWV) to determine if apoB would improve CVD-risk stratification, especially in type 1 diabetes adolescents with borderline LDL-C (100-129mg/dL). We hypothesized that type 1 diabetes adolescents with borderline LDL-C and elevated apoB (≥ 90 mg/dL) would have increased PWV compared to those with borderline LDL-C and normal apoB (<90 mg/dL), and that apoB would explain more of the variability of PWV than alternative lipid indices.

Methods—Fasting lipids, including apoB, were collected in 267 adolescents, age 12-19 years, with diabetes-duration >5 years and HbA1c $8.9 \pm 1.6\%$. Triglyceride to HDL-C ratio (TG/HDL-C)

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Ethical standards: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent and assent were obtained from all patients included in the study.

Conflict of interest: The authors declare that they have no conflict of interest.

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and nonHDL-cholesterol (nonHDL-C) were calculated. PWV was measured in the carotid-femoral segment.

Results—ApoB, nonHDL-C and TG/HDL-C correlated with PWV ($p<0.0001$). ApoB, nonHDL-C and TG/HDL-C remained significantly associated with PWV in fully-adjusted models. In adolescents with borderline LDL-C ($n=61$), PWV was significantly higher in those with elevated apoB than in those with normal apoB (5.6 ± 0.6 vs. 5.2 ± 0.6 m/s, $p<0.01$), and also remained significant after adjustment for CVD-risk factors ($p=0.0002$). Moreover, in those with borderline LDL-C, apoB explained more of the variability of PWV than nonHDL-C and TG/HDL-C.

Conclusion—Elevated apoB is associated with increased arterial stiffness in type 1 diabetes adolescents. Measurement of apoB in addition to LDL-C may be helpful in stratifying CVD-risk in type 1 diabetes adolescents, especially in those with borderline LDL-C.

Keywords

diabetes mellitus; lipids and lipoproteins; arterial stiffness

Introduction

Cardiovascular disease (CVD) is the primary cause of mortality in patients with type 1 diabetes (1). Dyslipidemia is one of the major contributory factors for CVD in type 1 diabetes (2-4). It is increasingly recognized that atherosclerosis begins early in life in association with dyslipidemia and tracks into adulthood (5; 6). Almost a third of children and adolescents with type 1 diabetes demonstrate dyslipidemia (2). Despite the robust evidence of dyslipidemia as a major risk factor in the development of CVD, the literature suggests significant under-treatment of this risk factor in children and adolescents with type 1 diabetes (2; 3; 7). Only 65% of participants of the T1D Exchange study met the American Diabetes Association's (ADA) targets for LDL-C (8).

Low-density lipoprotein cholesterol (LDL-C) remains the primary lipid marker of CVD risk in adolescents and adults with type 1 diabetes (5; 6; 9; 10). Data from the SEARCH for Diabetes in Youth (SEARCH) study showed that approximately one third of youth with type 1 diabetes had LDL-C in the borderline range (100-129 mg/dL) (7), which is associated with less clear CVD-risk and more ambiguous treatment recommendations. Studies have shown that apolipoprotein B (apoB) may add to current predictors of CVD and peripheral arterial disease (11-13). The SEARCH Case Control study showed that apoB levels were elevated in type 1 diabetes compared to non-diabetic controls independent of HbA1c or LDL-C (12; 14). For that reason, measurement of apoB in addition to LDL-C may help reclassify CVD risk in type 1 diabetes adolescents with borderline LDL-C levels and also determine when lipid lowering treatment should be considered.

Accordingly, our aim of this study was to investigate whether apoB was associated with pulse wave velocity (PWV), a measure of arterial stiffness that has been shown to be a strong predictor of CVD (15), in adolescents with type 1 diabetes. We hypothesized that there would be an independent association between apoB and PWV. Second, we hypothesized that among adolescents with type 1 diabetes and borderline LDL-C (100-129

mg/dl), patients with elevated apoB (≥ 90 mg/dL) would have higher PWV compared to those with normal apoB levels (<90 mg/dL). Finally, we hypothesized that apoB would explain more variability of PWV than non-HDL-cholesterol (nonHDL-C) and the ratio of triglycerides to HDL-C (TG/HDL-C) in adolescents with type 1 diabetes and borderline LDL-C.

Patients and Methods

The Determinants of Macrovascular Disease in Adolescents with Type 1 Diabetes study was initiated to investigate atherosclerotic disease risk in youth with type 1 diabetes (16). The study enrolled subjects aged 12-19 years from 2008 to 2010, with type 1 diabetes. Study participants with type 1 diabetes were diagnosed by islet cell antibody or by provider clinical diagnosis, had diabetes duration >5 years at entry into the study, and received care at the Barbara Davis Center for Childhood Diabetes. All subjects ($n=267$) with type 1 diabetes and data available for fasting lipids, apoB and PWV were included in this analysis. The study was approved by the Colorado Multiple Institution Review Board, and informed consent and assent (for subjects <18 years) were obtained from all subjects.

Pubertal status was evaluated by Tanner staging by a study investigator or clinical provider (17). After subjects had been laying supine for a minimum of 5 minutes, blood pressure measurements were obtained using a Dynapulse 5200A (Pulse Metric, San Diego, California), and 3 measurements were averaged. Pulse pressure calculated as the difference between the systolic blood pressure (SBP) and diastolic pressure (DBP). Height was measured to the nearest 0.1 cm with shoes removed using a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg using a Detecto scale (Detecto, Webb City, Missouri). BMI z-score was calculated using the 2000 Centers for Disease Control and Prevention growth chart standards. Participants were asked about smoking status, and current smoking is included in the analysis.

All subjects fasted overnight (≥ 8 hours) and were asked to refrain from caffeine intake and smoking within 8 hours prior to the study visit (due to potential effects on vascular measures). Urine samples were collected and urine creatinine and albumin were measured (RIA, Diagnostic Products). Microalbuminuria was defined as albumin-creatinine ratio (ACR) ≥ 30 mg/g. HbA1c was measured on the DCA Advantage by Siemens (Princeton, New Jersey) at the Children's Hospital Colorado main clinical lab. Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were performed in the Clinical Translational Research Core lab using a Beckman Coulter AU system (Beckman Coulter Inc, Brea, CA). LDL-C was calculated using the Friedwald formula (no subjects with TG >400 mg/dL) and apoB was measured by Beckman Array Nephelometer (Beckman Coulter Inc, Brea, CA) with an intra- and inter-assay coefficients of variation of 1.1 and 5.4% respectively. NonHDL-C was calculated by subtracting HDL-C from total cholesterol, and the ratio of TG to HDL-C was calculated by dividing TG by HDL-C. LDL-C levels were defined as elevated (≥ 130 mg/dL), borderline (100-129 mg/dL) and normal (<100 mg/dL) based on American Diabetes Association (ADA) guidelines (18). Elevated nonHDL-C was defined as ≥ 130 mg/dL and normal nonHDL-C was defined as <130 mg/dL, and

elevated apoB was defined as ≥ 90 mg/dL and normal apoB was defined as <90 mg/dL according to the ADA and American College of Cardiology (ACC) position statement (19).

Pulse wave velocity (PWV) was measured in the carotid-femoral segment using arterial tonometry with the Sphygmocor Vx (AtCor Medical, Lisle, IL). The carotid to femoral path length was measured from the reference point of the lowest portion of the sternal notch to the femoral pulse. The average of three measurements was entered into the SphygmoCor software. While recording a 3-lead ECG, pulse wave was recorded using arterial tonometry, first at carotid, followed by recording of pulse wave at femoral. The two pulse waves are subsequently compared using the R-wave as a reference, allowing us to compare the time from the R-wave to the foot of pulse waves, and to calculate PWV in meters per second (m/s). PWV measures were repeated in a subset of subjects to measure variability in the measure. For the 15 subjects with quality control data (repeated PWV measurements) at baseline, strong intraclass correlations were observed between the three PWV measures with an intraclass correlation coefficient (ICC) calculated at 0.91 (20).

Statistical analysis

Analyses were performed in SAS (version 9.3 or higher; SAS Institute, Cary, NC). Demographic and clinical characteristics among type 1 diabetes adolescents with normal, borderline and elevated LDL-C were compared using ANOVA for parametric variables and Kruskal-Wallis for nonparametric variables (Table 1). Furthermore, characteristics in type 1 diabetes adolescents with borderline LDL-C were compared in those with normal apoB levels to those with elevated apoB using Chi-Square tests for parametric categorical variables and *t*-test for continuous variables (Table 2). Wilcoxon Rank Sum was employed for TG and TG/HDL-C due to their non-parametric distribution. TG and TG/HDL-C were natural log-transformed for further analyses. Pearson correlations were performed to calculate correlation coefficients for CVD-risk factors (age, gender, BMI-z, HbA1c, HDL-C, LDL-C, lnTG, lnTG/HDL-C, apoB, nonHDL-C, DBP, SBP, ACR and smoking status) and PWV.

Multivariable linear regression models were employed to determine which variable remained significantly associated with PWV. Variables included in the multivariable models were based on *a priori* criteria: significance in previous work, significant contribution to the model (p-value of <0.1), or confounding between the main variable of interest and the outcome by $>10\%$. In addition to including apoB, nonHDL-C and lnTG/HDL-C, one at a time, the following variables were included in the fully adjusted models: age, sex, Tanner stage, BMI z-score, HbA1c, SBP, DBP and smoking status. We also re-ran the models excluding the 3 subjects on lipid lowering medications. We included squared partial correlation coefficients and tolerance to further examine the proportion of the variance of PWV explained by the independent variables. We also ran similar models exploring the associations between apoB, nonHDL-C and TG/HDL-C respectively with PWV in adolescents with borderline LDL-C.

We compared age, sex and Tanner stage-adjusted PWV for subjects divided into four groups: (1) subjects with normal LDL-C and normal apoB; (2) subjects with borderline LDL-C and normal apoB; (3) subjects with borderline LDL-C and elevated apoB; and (4)

subjects with elevated LDL-C and apoB (Figure 1). ANOVA tests were used to calculate adjusted least square means $\pm$ SD of PWV stratified by LDL-C and apoB groups.

Data are presented as means $\pm$ SD for continuous variables, median and IQR for continuous variables with non-parametric distribution, or count and percent for categorical variables. Correlation data are presented as r-coefficient, linear regression data are presented as $\beta \pm$ SE and squared partial correlation data are presented as R^2 . Significance was based on an α -level of 0.05.

Results

Subject characteristics stratified by LDL-C concentration for adolescents with type 1 diabetes are shown in Table 1. Most subjects were post-pubertal, with a mean age of 15.4 ± 2.2 years, and 80% of subjects had Tanner stage 4-5. PWV was positively correlated with apoB ($r=0.31$, $p<0.0001$), nonHDL-C ($r=0.31$, $p<0.0001$), $\ln$ TG/HDL-C ($r=0.27$, $p<0.0001$) and LDL-C ($r=0.29$, $p<0.0001$). PWV was also correlated with age ($r=0.38$, $p<0.0001$), sex ($r=0.16$, $p<0.01$ [sex=male]), $\ln$ TG ($r=0.26$, $p<0.0001$), HbA1c ($r=0.13$, $p=0.03$), DBP ($r=0.52$, $p<0.0001$), SBP ($r=0.49$, $p<0.0001$), pulse pressure ($r=0.12$, $p=0.049$), BMI z-score ($r=0.14$, $p=0.02$) and smoking status ($r=0.15$, $p=0.02$), but not HDL-C ($p=0.10$) and ACR ($p=0.34$).

PWV was significantly higher in subjects with elevated apoB compared to those with normal apoB levels (5.7 ± 0.7 vs. 5.2 ± 0.6 m/s, $p<0.0001$). Subjects with elevated apoB were also older than subjects with normal apoB (16.0 ± 2.1 vs. 15.2 ± 2.1 years, $p=0.02$), had higher HbA1c ($10.1 \pm 1.8\%$ vs. $8.6 \pm 1.3\%$, $p<0.0001$), higher LDL-C (121 ± 29 vs. 79 ± 17 mg/dL, $p<0.0001$), higher TG (108 vs. 69 mg/dL, $p<0.0001$), higher DBP (72 ± 6 vs. 67 ± 6 mm Hg, $p<0.0001$) and higher SBP (116 ± 8 vs. 113 ± 9 , $p=0.02$). The difference in PWV remained significant after adjusting for Tanner stage, age, sex, DBP, BMI z-score and HbA1c (5.5 ± 0.6 vs. 5.2 ± 0.6 m/s, $p<0.0001$).

Of the adolescents with borderline LDL-C ($n=61$), 57% ($n=35$) had elevated apoB. For these subjects, PWV was significantly higher compared to those with normal apoB levels ($n=26$) (5.6 ± 0.6 vs. 5.2 ± 0.6 m/s, $p<0.01$, Table 2). Subjects with borderline LDL-C and elevated apoB had similar age, sex, and BMI z-score distribution and higher HbA1c, total cholesterol, LDL-C and TG than those with borderline LDL-C and normal apoB levels (Table 2).

When we examined age, sex and Tanner stage adjusted PWV by LDL-C and apoB in adolescents with type 1 diabetes (Figure 1), subjects with normal LDL-C and normal apoB had similar mean PWV compared to subjects with borderline LDL-C and normal apoB (5.2 ± 1.0 vs. 5.0 ± 0.6 m/s, $p=0.34$). In contrast, subjects with borderline LDL-C and elevated apoB had significantly higher PWV (5.6 ± 0.7 m/s) than either of the groups with normal apoB ($p<0.0001$). Subjects with elevated LDL-C and apoB had the highest mean PWV (5.9 ± 0.6 m/s), but there was no significant difference in PWV between subjects with borderline LDL-C and elevated apoB and subjects with elevated LDL-C and elevated apoB ($p=0.08$).

In separate linear regression models, apoB ($\beta \pm \text{SE}$: 0.006 ± 0.002 , $p=0.0002$), nonHDL-C ($\beta \pm \text{SE}$: 0.005 ± 0.001 , $p=0.0001$) and $\ln \text{TG/HDL-C}$ ($\beta \pm \text{SE}$: 0.17 ± 0.07 , $p=0.01$) individually remained significantly associated with PWV after adjusting for age, sex, Tanner stage, BMI z-score, SBP, DBP, HbA1c and smoking status (Table 3). In subjects with borderline LDL-C, the association between apoB and PWV ($p=0.01$) also remained significant in a fully adjusted multivariable model, but not for nonHDL-C ($p=0.09$) and $\ln \text{TG/HDL-C}$ ($p=0.15$, Table 3). Furthermore, apoB ($R^2 = 13\%$) explained more of the variability of PWV in subjects with borderline LDL-C than nonHDL-C ($R^2 = 6\%$), TG/HDL-C ($R^2 = 4\%$) and conventional risk factors like HbA1c ($R^2 = 4\%$), SBP ($R^2 = 3\%$), DBP ($R^2 = 8\%$). We also reran the models without the 3 subjects on statins which did not change the significance of any of our findings.

Based on the above data, apoB appears to be more strongly related to PWV than nonHDL-C and $\ln \text{TG/HDL-C}$ in adolescents with borderline LDL-C. For that reason, with PWV as a surrogate for CVD-risk, 43% of the adolescents with borderline LDL-C could be reclassified as *low risk* based on normal levels of apoB, and the remaining 57% with elevated apoB could be classified as *high risk* for CVD (Figure 2).

Discussion

In the cohort we examined, almost one fourth of adolescents with type 1 diabetes had borderline LDL-C and of those, over half had elevated apoB. Those with elevated apoB demonstrated significantly higher arterial stiffness as measured by PWV, and the association between apoB and PWV remained significant after adjusting for other CVD-risk factors. Moreover, apoB was a stronger determinant of PWV than nonHDL-C, $\ln \text{TG/HDL-C}$ and conventional risk factors in adolescents with type 1 diabetes and borderline LDL-C in our study. This suggests that measurement of apoB in addition to LDL-C may be helpful to better stratify CVD-risk in adolescents with type 1 diabetes and borderline LDL-C, where CVD-risks and treatment recommendations are less clear.

PWV has emerged as a useful tool to evaluate vascular health (15; 21; 22) and a predictor of future CV events and all-cause mortality (15). It has been reported that children and adolescents with type 1 diabetes have increased arterial stiffness compared to healthy controls (23). It has been demonstrated that apoB at baseline is predictive of PWV measured 6 years later in healthy young adults (11), but no studies to date have examined the association between apoB and PWV in adolescents with type 1 diabetes.

There are extensive data demonstrating a relationship between LDL-C and atherosclerosis, but focusing only on LDL-C may not be adequate for patients with type 1 diabetes. A significant proportion of adult type 1 diabetes patients with CVD have LDL-C in the normal range (24; 25). One possible explanation for this discrepancy is the mismatch that has been described between the LDL-C and the number of atherogenic lipid particles. Moreover, LDL-C, nonHDL-C and apoB carry unique lipid information (26). LDL-C represents the cholesterol content of LDL, intermediate-density lipoprotein, and lipoprotein (a), whereas nonHDL-C is the LDL cholesterol and cholesterol content of circulating VLDL. ApoB directly measures the aggregate number of all atherogenic lipoproteins since each

atherogenic particle contains one apoB molecule. Therefore apoB may provide a more complete picture of the lipoprotein profile as it will account for small, dense, more atherogenic particles (27). Elevated apoB is also one of the most frequent lipid abnormalities in children and adolescents with type 1 diabetes (14).

The associations of ApoB and nonHDL-C with CVD-risk are well-described in the literature, but are not without controversy. Most studies, but not all (28; 29), have demonstrated that apoB and nonHDL-C are more closely associated with CVD-risk and all-cause mortality in adults than LDL-C (30-34). Moreover, TG/HDL-C has been shown to be an independent determinant of arterial stiffness in obese youth (35). It remains unclear however whether apoB is superior to nonHDL-C and TG/HDL-C for CVD-risk prediction (29; 36; 37), but our data suggest that apoB is more strongly related to PWV in adolescents with type 1 diabetes and borderline LDL-C. Consistent with our findings, Mora et al. recently reported in the Women's Health Study, that when LDL-C and apoB were discordant LDL-C under- or overestimated coronary risk by 20-50%, and the coronary risk was greatest when apoB and LDL-C were concordant (both greater or equal to median levels of cohort) (38).

The Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and the ADA do not yet recommend apoB for universal lipid screening in children and adolescents with or without type 1 diabetes (18; 39). Previous publications have suggested that apoB and other cholesterol indices should be considered to be complementary rather than competitive indices in adults with type 1 diabetes (40), and that it is useful to measure both apoB and LDL-C, especially when considering lipid lowering therapy (41). ApoB and LDL-C have been shown to be most concordant at their extremes (the highest and lowest quintiles of each) (42), so the use of both may be especially useful for values that are borderline.

There are important limitations to the present study worth mentioning. This study had an observational design and cross-sectional analysis without CVD endpoints (e.g. myocardial infarction) which would have been difficult to obtain in our adolescent cohort unless the participants were followed up for several decades. Although we adjusted for a variety of important confounding variables, we cannot rule out the presence of unknown risk factors that may have biased the present analyses. There are generally no standardized cut-offs for elevated PWV in the pediatric and adolescent age groups, and without a dichotomous outcome we were unable to perform conventional prediction performance analyses (e.g. C-statistics). Our cohort is also predominantly non-Hispanic white (81%), but this is consistent with the type 1 diabetes population in Colorado. The majority of our subjects were post-pubertal (80%) implying that our findings may not be applicable to pre-pubertal adolescents with type 1 diabetes; however, pharmacologic treatment of dyslipidemia in pre-pubertal children has been reserved for extremely elevated LDL-C. Adjusting for Tanner stage did not change the association between apoB and PWV. Furthermore, the lack of a significant difference in PWV between subjects with borderline LDL-C and elevated apoB and subjects with elevated LDL-C and apoB may be due to our limited sample size. While an association between apoB and PWV was observed, the relationship of apoB with future changes in arterial stiffness is unknown. Finally, as only 3 subjects were taking statins, we did not have

sufficient power for any meaningful analysis with statin use, but excluding these subjects from our analyses did not change the significance of our findings. While these data suggest that apoB may help stratify risk and identify patients who might benefit from statin therapy, such treatment remains rare in the adolescent T1D population.

In summary, dyslipidemia is common in children and adolescents with type 1 diabetes, and contributes to increased risk of developing CVD. The literature indicates that dyslipidemia is under-treated in children and adolescents with type 1 diabetes (2; 3; 7), which may be partly due to a significant proportion of subjects in this age group having borderline LDL-C where CVD-risks are less clear. We report significantly higher arterial stiffness in adolescents with type 1 diabetes, borderline LDL-C (100-129 mg/dL) and elevated apoB (≥ 90 mg/dL) than those with borderline LDL-C and normal apoB. Moreover, the association between apoB and PWV appears to be independent of other known CVD-risk factors. One potential means of improving CVD outcomes in type 1 diabetes is more precise estimation of dyslipidemia. Evaluation of apoB in adolescents with type 1 diabetes and borderline LDL-C could lead to better risk stratification and more aggressive treatment with lipid lowering medications in those in the highest risk category. Further research is needed, particularly longitudinal studies, to determine if apoB should be added to current lipid screening recommendations for adolescents with type 1 diabetes.

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References

1. Federation ID. Diabetes Atlas. 2007
2. Schwab KO, Doerfer J, Hecker W, Grulich-Henn J, Wiemann D, Kordonouri O, Beyer P, Holl RW. Spectrum and prevalence of atherogenic risk factors in 27,358 children, adolescents, and young adults with type 1 diabetes: cross-sectional data from the German diabetes documentation and quality management system (DPV). *Diabetes Care*. 2006; 29:218–225. [PubMed: 16443863]
3. Margeisdottir HD, Larsen JR, Brunborg C, Overby NC, Dahl-Jorgensen K. High prevalence of cardiovascular risk factors in children and adolescents with type 1 diabetes: a population-based study. *Diabetologia*. 2008; 51:554–561. [PubMed: 18196217]
4. Bjornstad P, Wadwa RP. Risks and benefits of statin use in young people with type 1 diabetes. *Curr Diab Rep*. 2014; 14:499. [PubMed: 24796934]
5. Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman WP 3rd, Herderick EE, Cornhill JF. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. *JAMA : the journal of the American Medical Association*. 1999; 281:727–735.
6. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *The New England journal of medicine*. 1998; 338:1650–1656. [PubMed: 9614255]

7. Kershner AK, Daniels SR, Imperatore G, Palla SL, Petitti DB, Pettitt DJ, Marcovina S, Dolan LM, Hamman RF, Liese AD, Pihoker C, Rodriguez BL. Lipid abnormalities are prevalent in youth with type 1 and type 2 diabetes: the SEARCH for Diabetes in Youth Study. *The Journal of pediatrics*. 2006; 149:314–319. [PubMed: 16939739]
8. Wood JR, Miller KM, Maahs DM, Beck RW, Dimeglio LA, Libman IM, Quinn M, Tamborlane WV, Woerner SE. Most Youth With Type 1 Diabetes in the T1D Exchange Clinic Registry Do Not Meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes Clinical Guidelines. *Diabetes Care*. 2013; 36:2035–2037. [PubMed: 23340893]
9. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. *Circulation*. 2001; 104:2815–2819. [PubMed: 11733400]
10. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, Jarvisalo MJ, Uhari M, Jokinen E, Ronnema T, Akerblom HK, Viikari JS. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA : the journal of the American Medical Association*. 2003; 290:2277–2283.
11. Koivisto T, Hutri-Kahonen N, Juonala M, Koobi T, Aatola H, Lehtimäki T, Viikari JS, Raitakari OT, Kahonen M. Apolipoprotein B is related to arterial pulse wave velocity in young adults: the Cardiovascular Risk in Young Finns Study. *Atherosclerosis*. 2011; 214:220–224. [PubMed: 21122858]
12. Albers JJ, Marcovina SM, Imperatore G, Snively BM, Stafford J, Fujimoto WY, Mayer-Davis EJ, Petitti DB, Pihoker C, Dolan L, Dabelea DM. Prevalence and determinants of elevated apolipoprotein B and dense low-density lipoprotein in youths with type 1 and type 2 diabetes. *The Journal of clinical endocrinology and metabolism*. 2008; 93:735–742. [PubMed: 18089692]
13. Mancera-Romero J, Sanchez-Chaparro MA, Rioja J, Ariza MJ, Olivecrona G, Gonzalez-Santos P, Valdivielso P. Fasting apolipoprotein B48 is a marker for peripheral arterial disease in type 2 diabetes. *Acta diabetologica*. 2013; 50:383–389. [PubMed: 23053881]
14. Guy J, Ogden L, Wadwa RP, Hamman RF, Mayer-Davis EJ, Liese AD, D'Agostino R Jr, Marcovina S, Dabelea D. Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. *Diabetes Care*. 2009; 32:416–420. [PubMed: 19092167]
15. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *Journal of the American College of Cardiology*. 2010; 55:1318–1327. [PubMed: 20338492]
16. Brown TL, Snell-Bergeon J, Maahs D, Bishop FK, Prentice N, Aragon B, Wadwa P. Loss of Female Cardiovascular Protection in Type 1 Diabetes May Begin in Adolescence. *Diabetes*. 2011; 60(Suppl 1)
17. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Archives of disease in childhood*. 1970; 45:13–23. [PubMed: 5440182]
18. Standards of medical care in diabetes--2013. *Diabetes Care*. 2013; 36(Suppl 1):S11–66. [PubMed: 23264422]
19. 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. *Journal of the American College of Cardiology*. 2008; 51:1512–1524. [PubMed: 18402913]
20. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychological bulletin*. 1979; 86:420–428. [PubMed: 18839484]
21. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, Eber B. Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation*. 2004; 109:184–189. [PubMed: 14662706]
22. Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, Jacobson M, Mahoney L, Mietus-Snyder M, Rocchini A, Steinberger J, McCrindle B. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. *Hypertension*. 2009; 54:919–950. [PubMed: 19729599]

23. Haller MJ, Samyn M, Nichols WW, Brusko T, Wasserfall C, Schwartz RF, Atkinson M, Shuster JJ, Pierce GL, Silverstein JH. Radial artery tonometry demonstrates arterial stiffness in children with type 1 diabetes. *Diabetes Care*. 2004; 27:2911–2917. [PubMed: 15562206]
24. Sachdeva A, Cannon CP, Deedwania PC, Labresh KA, Smith SC Jr, Dai D, Hernandez A, Fonarow GC. Lipid levels in patients hospitalized with coronary artery disease: an analysis of 136,905 hospitalizations in Get With The Guidelines. *American heart journal*. 2009; 157:111–117 e112. [PubMed: 19081406]
25. Libby P. The forgotten majority: unfinished business in cardiovascular risk reduction. *Journal of the American College of Cardiology*. 2005; 46:1225–1228. [PubMed: 16198835]
26. Martin SS, Michos ED. Are we moving towards concordance on the principle that lipid discordance matters? *Circulation*. 2014; 129:539–541. [PubMed: 24345401]
27. Barter PJ, Ballantyne CM, Carmena R, Castro Cabezas M, Chapman MJ, Couture P, de Graaf J, Durrington PN, Faergeman O, Frohlich J, Furberg CD, Gagne C, Haffner SM, Humphries SE, Jungner I, Krauss RM, Kwiterovich P, Marcovina S, Packard CJ, Pearson TA, Reddy KS, Rosenson R, Sarrafzadegan N, Sniderman AD, Stalenhoef AF, Stein E, Talmud PJ, Tonkin AM, Walldius G, Williams KM. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. *Journal of internal medicine*. 2006; 259:247–258. [PubMed: 16476102]
28. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, Patsch W. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. 2001; 104:1108–1113. [PubMed: 11535564]
29. Bao W, Srinivasan SR, Berenson GS. Tracking of serum apolipoproteins A-I and B in children and young adults: the Bogalusa Heart Study. *Journal of clinical epidemiology*. 1993; 46:609–616. [PubMed: 8326345]
30. Ramjee V, Sperling LS, Jacobson TA. Non-high-density lipoprotein cholesterol versus apolipoprotein B in cardiovascular risk stratification: do the math. *Journal of the American College of Cardiology*. 2011; 58:457–463. [PubMed: 21777740]
31. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner 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. [PubMed: 11755609]
32. McQueen MJ, Hawken S, Wang X, Ounpuu S, Sniderman A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, Kazmi K, Yusuf 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. [PubMed: 18640459]
33. Albers JJ, Slee A, O'Brien KD, Robinson JG, Kashyap ML, Kwiterovich PO Jr, Xu P, Marcovina SM. Relationship of Apolipoproteins A-1 and B, and Lipoprotein(a) to Cardiovascular Outcomes: The AIM-HIGH Trial (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglyceride and Impact on Global Health Outcomes). *Journal of the American College of Cardiology*. 2013; 62:1575–1579. [PubMed: 23973688]
34. Juonala M, Viikari JS, Kahonen M, Solakivi T, Helenius H, Jula A, Marniemi J, Taittonen L, Laitinen T, Nikkari T, Raitakari OT. Childhood levels of serum apolipoproteins B and A-I predict carotid intima-media thickness and brachial endothelial function in adulthood: the cardiovascular risk in young Finns study. *Journal of the American College of Cardiology*. 2008; 52:293–299. [PubMed: 18634985]
35. Urbina EM, Khoury PR, McCoy CE, Dolan LM, Daniels SR, Kimball TR. Triglyceride to HDL-C ratio and increased arterial stiffness in children, adolescents, and young adults. *Pediatrics*. 2013; 131:e1082–1090. [PubMed: 23460684]
36. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes 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 : the journal of the American Medical Association*. 2007; 298:776–785.
37. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and

- risk of vascular disease. JAMA : the journal of the American Medical Association. 2009; 302:1993–2000.
38. Mora S, Buring JE, Ridker PM. Discordance of Low-Density Lipoprotein (LDL) Cholesterol With Alternative LDL-Related Measures and Future Coronary Events. *Circulation*. 2014; 129:553–561. [PubMed: 24345402]
39. Expert Panel on Integrated Guidelines for Cardiovascular H, Risk Reduction in C, Adolescents, National Heart L, Blood I. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics*. 2011; 128(Suppl 5):S213–256. [PubMed: 22084329]
40. Mazanderani AB, Wise SJ, Tildesley HD. Apolipoprotein B levels in adults with type 1 diabetes not receiving lipid-lowering therapy. *Clinical biochemistry*. 2009; 42:1218–1221. [PubMed: 19497316]
41. Sniderman AD, St-Pierre AC, Cantin B, Dagenais GR, Despres JP, Lamarche B. Concordance/discordance between plasma apolipoprotein B levels and the cholesterol indexes of atherosclerotic risk. *The American journal of cardiology*. 2003; 91:1173–1177. [PubMed: 12745098]
42. Sniderman A, Williams K, Cobbaert C. ApoB versus non-HDL-C: what to do when they disagree. *Current atherosclerosis reports*. 2009; 11:358–363. [PubMed: 19664379]

Figure 1. Age, sex and Tanner-adjusted PWV in adolescents with type 1 diabetes by LDL-C and apoB

Age, sex and Tanner stage adjusted means for PWV in adolescents with normal LDL-C and apoB, borderline LDL-C and normal apoB, borderline LDL-C and elevated apoB and elevated LDL-C and elevated apoB. Adjusted PWV in adolescents with borderline LDL-C and normal apoB was not significantly different from those with normal LDL-C and normal apoB ($p=0.34$). Adjusted PWV in adolescents with borderline LDL-C and elevated apoB was significantly higher than in those with borderline LDL-C and normal apoB ($p<0.0001$). The difference between in adjusted PWV between adolescents with elevated LDL-C and apoB compared to those with borderline LDL-C and elevated apoB was not statistically significant ($p=0.08$)

Type 1 diabetes adolescent subjects		LDL-C		
		<100 mg/dL (Low Risk)	100-129 mg/dL (Intermediate Risk)	≥130 mg/dL (High Risk)
ApoB	<90 mg/dL	179 (94%)	26 (43%)	0 (0%)
	≥90 mg/dL	12 (6%)	35 (57%)	15 (100%)

Figure 2. Reclassification of CVD-risk in type 1 diabetes adolescents with borderline LDL-C
 Adolescents with type 1 diabetes were grouped according to LDL-C (normal, borderline and elevated) and apoB (normal and elevated). Approximately one-quarter of participants had borderline LDL-C, and of those participants 43% had normal apoB and not significantly different adjusted PWV to those with normal LDL-C/normal apoB (Figure 1). Similarly, 57% of the participants with borderline LDL-C had elevated apoB and not significantly different adjusted PWV from those with elevated LDL-C/elevated apoB (Figure 1). Using PWV as a surrogate for cardiovascular disease (CVD), apoB can be used to stratify CVD risk in adolescents with type 1 diabetes and borderline LDL-C.

Table 1
Clinical characteristics of adolescents with type 1 diabetes

Variables	Normal LDL (n=191)	Borderline LDL (n=61)	Elevated LDL (n=15)	p-value
Age (years)	15.2±2.1	15.7±2.3	16.5±1.6	0.04
Race-Ethnicity (% NHW)	82%	82%	67%	0.35
Type 1 diabetes Duration (years)	8.6±3.0	9.0±2.8	8.8±3.6	0.61
BMI (z-score)	0.6±0.7	0.6±0.8	0.8±0.8	0.48
Tanner stage (% 4-5)	80%	77%	91%	0.37
Current Smokers (%)	8%	7%	7%	0.94
HbA1c (%)	8.7±1.4	9.2±1.5	11.0±2.1	<0.0001
Total cholesterol (mg/dL)	142±20	184±16	245±43	<0.0001
LDL-C (mg/dL)	76±14	112±9	157±32	<0.0001
*TG (mg/dL)	66 (53-86)	81 (69-106)	120 (102-176)	<0.0001
*TG/HDL-C (mg/mg)	1.4 (1.0-1.9)	1.6 (1.2-2.2)	2.1 (1.6-3.2)	0.0002
HDL-C (mg/dL)	50±10	53±11	57±11	0.01
ApoB (mg/dL)	67±14	94±13	137±33	<0.0001
NonHDL-C (mg/dL)	92±17	131±12	188±40	<0.0001
*ACR (mg/g)	6.9 (4.3-12.0)	7.6 (4.8-15.9)	10.8 (5.7-27.3)	0.14
DBP(mm Hg)	68±6	69±6	76±7	<0.0001
SBP (mm Hg)	112±9	113±8	116±6	0.37
PWV (m/s)	5.2±0.6	5.4±0.6	6.0±0.9	<0.0001

* Median and interquartile range (IQR)

Table 2
Clinical characteristics of type 1 diabetes adolescents with borderline LDL-C and stratified by apoB

Variables	ApoB <90 mg/dL (n=26)	ApoB 90 mg/dL (n=35)	p-value
Age (years)	15.8 ± 2.4	15.6 ± 2.3	0.74
Sex (% male)	54	43	0.40
BMI (z-score)	0.5 ± 0.9	0.7 ± 0.8	0.40
Tanner stage (% 4-5)	77	77	0.98
Current smokers (%)	8	6	0.76
HbA1c (%)	8.5 ± 1.3	9.7 ± 1.5	0.004
Total cholesterol (mg/dL)	174 ± 8	192 ± 17	<0.0001
HDL-C (mg/dL)	52 ± 9	54 ± 12	0.55
LDL-C (mg/dL)	107 ± 7	116 ± 8	<0.0001
*TG (mg/dL)	72 (61-82)	94 (71-124)	0.002
*TG/HDL-C (mg/mg)	1.4 (1.1-1.8)	1.9 (1.2-2.4)	0.01
NonHDL-C (mg/dL)	121 ± 7	138 ± 10	<0.0001
*ACR (mg/g)	6.7 (3.5-8.8)	9.5 (6.4-18.6)	0.05
SBP (mmHg)	111 ± 6	115 ± 9	0.06
DBP (mmHg)	67 ± 5	70 ± 6	0.048
PWV (m/s)	5.2 ± 0.6	5.6 ± 0.6	<0.01

* Median and interquartile range (IQR)

Table 3
Separate multivariable linear regression models for PWV in adolescents with type 1 diabetes

Variables	$\beta \pm SE^a$	Squared partial correlation (R^2)	Tolerance	p-value
All subjects:				
ApoB *	0.006 $\pm$ 0.002	0.05	0.66	0.0002
Elevated apoB (90mg/dL) *	0.32 $\pm$ 0.09	0.05	0.80	0.0002
NonHDL-C *	0.005 $\pm$ 0.001	0.06	0.73	0.0001
Elevated nonHDL-C (130mg/dL) *	0.33 $\pm$ 0.09	0.05	0.84	0.0004
Ln TG/HDL-C *	0.17 $\pm$ 0.07	0.02	0.85	0.01
Subjects with borderline LDL-C (100-129mg/dL):				
ApoB *	0.02 $\pm$ 0.006	0.13	0.66	0.01
Elevated apoB (90mg/dL) *	0.45 $\pm$ 0.144	0.17	0.75	0.003
NonHDL-C *	0.01 $\pm$ 0.007	0.06	0.68	0.09
Elevated nonHDL-C (130mg/dL) *	0.33 $\pm$ 0.141	0.10	0.83	0.02
Ln TG/HDL-C *	0.28 $\pm$ 0.19	0.04	0.77	0.15

* Separate linear regression models adjusted for age, sex, tanner stage, BMI, SBP, DBP, HbA1c and current smoking

^a β -coefficient represents the difference in PWV for every 1-unit difference in the independent variable.