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Impact of Lipid Measurements in Youth in Addition to Conventional Clinic-Based Risk Factors on Predicting Preclinical Atherosclerosis in Adulthood: The International Childhood Cardiovascular Cohort (i3C) Consortium

Running Title: *Koskinen et al.; Assessment of CVD Risk in Youth on cIMT in Adults*

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Abstract

***Background*—**Data suggest that the prediction of adult cardiovascular disease using a model comprised entirely of adult non-laboratory based risk factors is equivalent to an approach that additionally incorporates adult lipid measures. We assessed and compared the utility of a risk model based solely on non-laboratory risk factors in adolescence vs. a lipid model based on non-laboratory risk factors + lipids for predicting high-risk carotid intima-media thickness (cIMT) in adulthood.

***Methods*—**The study comprised 2,893 participants aged 12-18 years from four longitudinal cohort studies from the United States (Bogalusa Heart Study and the Insulin Study), Australia (Childhood Determinants of Adult Health Study) and Finland (The Cardiovascular Risk in Young Finns Study) and followed into adulthood when cIMT was measured (mean follow-up 23.4 years). Overweight status was defined according to the Cole classification. Hypertension was defined according to the Fourth Report on High Blood Pressure in Children and Adolescents from the National High Blood Pressure Education Program. High-risk plasma lipid levels were defined according to the National Cholesterol Education Program (NCEP) Expert Panel on Cholesterol Levels in Children. High cIMT was defined as a study-specific value ≥90th percentile. Age-and sex were included in each model.

***Results*—**In univariate models all risk factors except for borderline high-and high triglycerides in adolescence were associated with high cIMT in adulthood. In multivariable models (RR [95% CI]), male sex (2.7 [2.0-2.6]), pre-hypertension (1.4 [1.0-1.9]), hypertension (1.9 [1.3-2.9]), overweight (2.0 [1.4-2.9]), obesity (3.7 [2.0-7.0]), borderline high LDL-cholesterol (1.6 [1.2-2.2]), high LDL-cholesterol (1.6 [1.1-2.1]) and borderline low HDL-cholesterol (1.4 [1.0-1.8]) remained significant predictors of high cIMT (P always < 0.05). The addition of lipids into the non-laboratory risk model slightly, but significantly, improved discrimination in predicting high cIMT compared with non-laboratory-based risk factors only (c-statistics for laboratory-based model 0.717 [95%CI 0.685-0.748] and for non-laboratory 0.698 [95%CI 0.667-0.731], P=0.02).

***Conclusions*—**Non-laboratory-based risk factors and lipids measured in adolescence independently predicted preclinical atherosclerosis in young adulthood. The addition of lipid measurements to traditional clinic based risk factor assessment provided a statistically significant but clinically modest improvement on adolescent prediction of high cIMT in adulthood.

Key Words: risk prediction; lipids; intima-media thickness

Clinical Perspective

What is new?

- Non-laboratory-based risk factors (sex, blood pressure status, body mass index status) and lipids (HDL- and LDL-cholesterol status) measured in adolescence independently predicted high carotid intima media thickness, a marker of preclinical atherosclerotic cardiovascular disease, in young adulthood.
- The addition of lipid measurements to a prediction model that only considered non-laboratory-based risk factors provided a clinically modest improvement in the ability to predict adolescents likely to develop high carotid intima media thickness in adulthood.

What are the clinical implications?

- In the absence of information on lipids, risk assessment using only non-laboratory-based risk factors might be a useful alternative in predicting adolescents at risk of developing adult preclinical atherosclerotic cardiovascular disease.
- Future research should determine the utility of multiple lipid and non-laboratory-based risk factor measurements collected throughout youth to the prediction of adult preclinical atherosclerotic cardiovascular disease.

Atherosclerosis is a multifactorial disease with its roots in childhood[1], suggesting that primary prevention of cardiovascular diseases would ideally be targeted to children and adolescents. Recent pediatric guidelines in cardiovascular disease (CVD) risk assessment recommend universal (population-wide) lipid screening in youth[2]. However, efforts to create a risk stratification system that could be implemented cost effectively and on a large scale, based on routine and easily collected measures, and without a need for additional laboratory testing. A study in adults[3] showed promising results supporting this kind of approach, with a non-laboratory-based risk model predicting cardiovascular events as accurately as one that relied on laboratory-based measurements.

Increased carotid intima-media thickness (cIMT), as assessed noninvasively by ultrasound, is a marker of structural atherosclerosis[4]. We and others have previously shown that elevated risk factors in youth predict greater cIMT in adulthood[1]. cIMT has also been shown to be an independent risk factor for cardiovascular events[5]. Thus, in the absence of cardiovascular events, measurements of cIMT by non-invasive imaging techniques provide a surrogate end-point to assess early atherosclerosis[4].

In this study, we use data from four cohort studies of cardiovascular risk factors initiated in childhood that have followed participants into adulthood: the Cardiovascular Risk in Young Finns Study, Finland[1], the Childhood Determinants of Adult Health Study, Australia[6], the Bogalusa Heart Study, USA.[7], and the Insulin Study, USA.[8,9]. Our aim was to compare risk prediction models based on non-laboratory vs. non-laboratory + lipids data obtained during adolescence for predicting high-risk cIMT in adulthood. For simplicity in the text we refer to the non-laboratory + lipids-model as a “lipid” model.

Methods

Study sample

Data were analyzed in 2,893 participants aged 12-18 years at baseline from four longitudinal cohort studies with mean follow-up of 23.4 years. Each study was approved by the appropriate institutional review boards, and written informed consent or assent was obtained from all the study participants over age 18, or assent from the participants and consent from their parents for participants under the age of 18. Risk factors in each cohort were measured as part of selective screening. None of the participants were using lipid lowering- or antihypertensive medication or diagnosed with type 2 diabetes at baseline. Participants with type 1 diabetes were excluded from the present study. The data, analytic methods, and study materials will not be made available to other researchers outside i3C consortium for purposes of reproducing the results or replicating the procedure. Researchers interested in the data, methods, or analysis can contact the corresponding author for more information.

Cardiovascular Risk in Young Finns Study (YFS)

The YFS sample has been previously described in detail[1]. YFS is an ongoing epidemiological cohort study. In 1980, 3596 children and adolescents aged 3, 6, 9, 12, 15, and 18 years participated in the first cross-sectional examination. Study participants were chosen randomly from national population registers from five Finnish university cities and their rural surroundings. The majority of subjects in the present study were from YFS, including 2,079 participants aged 12, 15, and 18 years at baseline (1986) and who had low density lipoprotein (LDL)- and high density lipoprotein (HDL)-cholesterol and smoking data at baseline and carotid artery ultrasonography data in 2001 (ages 27, 30 and 33) or 2007 (ages 33, 36 and 39). Data from

the most recent visit that included measurement of cIMT were used to maximize the follow-up period.

Childhood Determinants of Adult Health (CDAH)

The CDAH sample has been described in detail elsewhere[6]. CDAH baseline data were collected in 1985 on a representative sample of 8,498 school children 7 to 15 years of age as part of the Australian Schools Health and Fitness Survey. The present study included data from 272 participants, 12-15 years old at baseline, with risk factor data at baseline and carotid artery ultrasound measurements at 28 to 36 years of age (2004–06).

Bogalusa Heart Study (BHS)

The BHS sample has also been described in detail elsewhere[7]. The BHS is a biracial community-based investigation of the early natural history of CVD. For this analysis 436 participants aged 12-18 years at baseline who had risk factor data available from either the 1984-85 or 1987-88 cross-sectional surveys (baseline) and had measures including ultrasound data collected during 2001-10 (follow-up) were included. Data from the first and the most recent visits were used to maximize the follow-up period.

The Insulin Study (IS)

The Insulin Study (Minneapolis, Minnesota) has been described in detail elsewhere[8,9]. The initial cohort was selected randomly after blood pressure screening of 12,043 fifth- to eighth-grade public school children. This analysis included data on 106 participants who were measured between 2000-08 at the age of 17-18 years and who had adulthood risk factor measurements available at the age of 21-24 years.

Clinic measurements and smoking status

Height and weight were measured at all time points. Body mass index (BMI) was calculated using the formula: weight [kg] / height [m] ^{2}. A random zero sphygmomanometer was used to measure blood pressure (BP) in the YFS, BHS and IS. In CDAH, BP was measured with a standard mercury sphygmomanometer at childhood and a digital automatic monitor (Omron HEM907, Omron Healthcare Inc, Kyoto, Japan) at adulthood. In YFS the average of three measurements of the first and fifth Korotkoff sounds were used to define systolic and diastolic BP. The mean of two measurements was used in the CDAH, BHS and IS analyses. Venous blood samples were taken after a 12-hour fast from the antecubital vein. In YFS at baseline, serum cholesterol and triglycerides were measured using fully enzymatic Boehringer CHOD-PAP kits with an OLLI 3000 analyzer. Subsequently, an Olympus System reagent analyzer in a clinical chemistry analyzer (AU400, Olympus), was used to determine lipid levels. Serum HDL-cholesterol was measured by the dextran sulphate 500,000 method. In CDAH in 1985, serum total cholesterol and triglycerides were determined according to the Lipid Research Clinics Program and HDL-cholesterol was analyzed after precipitation of apolipoprotein-B–containing lipoproteins with heparin-manganese[10]. In BHS the HDL-cholesterol and triglycerides were measured using chemical procedures with a Technicon Auto Analyzer II (Technicon Instrument Corp, Tarrytown, NY), according to the laboratory manual of the Lipid Research Clinics Program[11]. LDL-cholesterol was calculated using the Friedewald formula[12]. Since baseline years, these variables were determined by enzymatic procedures using the Abbott VP instrument (Abbott Laboratories, North Chicago, IL)[13]. Serum concentrations of LDL- and HDL-cholesterol were analyzed by a combination of heparin-calcium precipitation and agar-agarose gel electrophoresis procedures. In IS serum lipids were analyzed in the University of Minnesota

laboratory with a Cobas FARA[14]. HDL-cholesterol was determined after precipitation of non-HDL lipoproteins with a dextran-sulfate magnesium precipitating reagent. Triglycerides were determined with a standard glycerol blanked enzymatic triglyceride method. LDL-cholesterol was calculated using the Friedewald formula in YFS, CDAH and IS[12]. The coefficient of variation (CV) for within-assay precision in YFS was 2.2% for total cholesterol, 2.3% for HDL-cholesterol and 3.8% for serum triglycerides. Both US cohorts and CDAH used chemical and enzymatic procedures meeting the performance requirements of the Lipid Clinics Program and Lipid Standardization Program of the Centers for Disease Control and Prevention, which routinely monitors the accuracy of measurements of total cholesterol, triglyceride and HDL-cholesterol concentrations. Participants were asked a series of questions about smoking at baseline. Details of these questionnaires are presented in online supplement. Responses were collapsed into a binomial categorical variable indicating: 1) regular smoking or at least five cigarettes per week (≥5/week) and. 2) no smoking or less than five cigarettes per week (<5/week). Physical activity was assessed by using a self-administered questionnaire at baseline in the YFS cohort only (see online supplement).

Carotid artery ultrasound studies

B-mode ultrasound studies of the left carotid artery were performed at follow-up examinations using standardized protocols for each study described in detail elsewhere[1,15-17]. In YFS, to assess intra-individual reproducibility of ultrasound measurements, 57 participants were re-examined 3 months after the initial visit. The average absolute difference and standard deviation between measurements was 0.05±0.04 mm. In CDAH, reproducibility for replicate maximum cIMT measurements was assessed in a random sample of 30 participants. The average absolute difference and SD was 0.02±0.04 mm. In BHS, 75 participants underwent repeat ultrasound

examinations 10-12 days after their initial visit to determine intra-individual reproducibility. The average absolute difference and standard deviation between measurements for all cIMT segments was 0.05±0.03 mm. In IS reproducibility of the cIMT showed a mean difference (±SD) of 0.02 ± 0.03 for analyses separated by one week.

Definition of high cIMT in adulthood

cIMT in adulthood was defined as ≥ 90th percentile for study-cohort and study-year specific values. In sensitivity analyses, similar results were found using cutpoints corresponding to the 80th cIMT percentiles (data not shown).

Definition of risk factors during adolescence

Due to generalizability of these data to the clinical setting and to be consistent with current recommendations, we analyzed the data by using categorical adolescent risk factors classified according to the recent recommendations from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents[2]. This enabled standardization among cohorts by adjusting to the same external standard while accounting for age-, sex-, and height- (where appropriate) specific growth patterns. Overweight status was defined according to the Cole classification of BMI[18]. Prehypertension or hypertension was defined according to the Fourth Report on High Blood Pressure in Children and Adolescents from the National High Blood Pressure Education Program[19]. High-risk plasma lipid levels were defined according to the National Cholesterol Education Program (NCEP) Expert Panel on Cholesterol Levels in Children[20]. However, due to differences in the range of risk factor levels among the cohorts, and changes in secular trends[21], the main analyses were also performed by using study- and visit-specific z-scores. The results were similar in terms of the direction of

effect and level of significance with results obtained from the main analyses. Detailed results and discussion from these analyses are presented in the text and in online supplement.

Statistical methods

Statistical analyses were performed with SAS 9.4. Statistical significance was inferred at a two-tailed value of $P \leq 0.05$. The normality assumptions of the residuals were assessed by examining histograms of the residuals and normal probability plots. The residuals were normally distributed. Values for plasma triglycerides were $\log_e$-transformed to correct for skewness. No significant interactions were observed between sex and adolescent risk factors with continuous ultrasound variables, indicating that the associations of risk markers and ultrasound variables were similar between sexes. Therefore, data from males and females were combined in all models. In the online sensitivity analyses, study-cohort and -year specific z-scores were constructed for each adolescent risk factor. Age- and sex adjusted ANOVA was used to compare differences in characteristics among study groups. Risk ratios and 95% confidence intervals were estimated using log binomial regression and used to examine associations between adolescent risk measurements and adulthood risk for high cIMT. Linear and polynomial regression models were used to examine the associations for the non-laboratory and lipid measurements with adult preclinical atherosclerosis. The ability of non-laboratory and lipid risk data in adolescence to predict high cIMT in adulthood was assessed using area under receiver-operating characteristic curves (AUC), category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) measures. Calibration was assessed by using the Hosmer-Lemeshow (H-Lχ2) goodness of fit test. Additionally, for each event using Poisson regression, we estimated the event probability under the non-laboratory model (age, sex, BMI, blood pressure, smoking) and under the lipid model (non-laboratory plus lipids). Improvement in

prediction probability (IPP) was estimated as the observed risk difference as a function of the reclassification probability, that is, the alternative probability minus base probability, which each probability was the individual solution to the whole regression equation (expected value of the mean for each individual). The logic of IPP is as follows. For fixed expected risk for an individual under the base model IPP, if the expected risk under the alternative model is less, the observed risk should be less; and if the expected risk under the alternative model is more, the observed risk should be greater. IPP was operationalized in two ways: first, the differential observed event rate for those whose reclassification probability was negative (classified to lower risk status) compared to those whose reclassification probability was positive (classified to higher risk status), and, second, the P-value of the regression coefficient of observed higher cIMT on the reclassification probability. In both methods, we also adjusted for base risk. Details of this method is described elsewhere[22].

Results

Baseline characteristics

Baseline risk factors stratified by study cohort, with age- and sex-adjusted differences, are shown in Table 1. The gender distribution was similar among study groups, but significant differences were observed for BP, BMI, lipids and smoking among the cohorts (all $P < 0.0002$).

Non-laboratory- vs Lipid (i.e. Non-laboratory with lipid measurements) based risk assessment in predicting high cIMT in adulthood

Univariate analyses (Table 2) assessing relations between risk factors and cIMT showed significant associations for age, sex and categorical risk factors (BMI, smoking, LDL- and HDL-cholesterol). Table 3 shows the results for a multivariable model assessing risk ratios for high

cIMT (≥90th percentile) in adulthood according to non-laboratory and lipid risk factors measured during adolescence. Among the non-laboratory risk factors sex, categorical BMI and categorical blood pressure were significantly associated with high cIMT. When lipids were introduced into the model, the significant association remained for sex, overweight, obesity, prehypertension-/hypertension, borderline-high -, and high LDL-cholesterol and borderline-low HDL. Similar associations were observed for study- and visit-specific continuous z-score risk factors (online supplemental tables 1 and 2).

Figure 1 shows the receiver operating characteristic (ROC) curves for non-laboratory model (blood pressure, BMI, smoking) and lipid model (non-laboratory plus lipids) prediction of adult cIMT ≥90th percentile. Addition of lipids to the non-laboratory model led to a higher C statistics: (0.688; 0.655-0.721) versus (0.701; 0.669-0.733), P for difference 0.038.
When study cohorts were analyzed separately, no significant differences between models were observed: for YFS the non-laboratory based AUC was 0.702 (0.665-0.739) and lipid based AUC 0.711 (0.675-0.747), P for difference 0.07. For CDAH the non-laboratory based AUC was 0.584 (0.452-0.717) and lipid based AUC 0.691 (0.543-0.839), P for difference 0.19. For BHS the non-laboratory based AUC was 0.706 (0.623-0.788) and lipid based AUC 0.716 (0.632-0.799), P for difference 0.59. For IS the non-laboratory based AUC 0.885 (0.796-0.974) and lipid based AUC 0.890 (0.789-0.99), P for difference 0.87.

Table 4 provides data to compare the utility of non-laboratory vs. lipid based risk assessment in predicting adult high cIMT. Adding lipids to the non-laboratory model significantly improved the AUC, IDI, and NRI values as shown in model 1 (P always <0.04). Model 2 shows the results when a comparison was made between non-laboratory and modified lipid models where BMI is removed from the lipid model. AUCs were now similar between the

models (in model 2) predicting high cIMT (P for difference 0.39). In addition, neither IDI nor NRI (0.007 and 0.002, P for difference always > 0.10) changed significantly with the lipid-based model compared to the non-laboratory based model (Table 4). Goodness-of-fit indicated by the Hosmer-Lemeshow $\chi 2$ was acceptable for all models examined. The IPP reclassification of high cIMT probability based on adding lipids to the base regression equation led to an improvement in prediction (Table 5). Cumulative incidence for high cIMT was 6.35 % in participants whose risk was reclassified downward by adding lipids versus 9.70 % in participants whose risk was reclassified upward with an absolute difference of 3.35 % (P=0.0021) which means an added discrimination of 43 % of the average risk for everyone. The cumulative risk does not account for time between risk factor and cIMT measurements. The Poisson model, which does account for elapsed time, showed similar results but a lower reclassification percentages compared to the linear model (5.59 % in participants whose risk was reclassified downward versus 8.09 % in participants whose risk was reclassified upward, P for difference 0.0078).

Figure 2 shows the incremental value of cumulative risk factor burden. Non-laboratory risks included overweight or obese, elevated blood pressure and smoking. Lipid risk factors additionally included elevated LDL- and triglyceride levels and low HDL-levels. Risk for developing high cIMT in adulthood was similar whether a participant had positive risk for all three non-laboratory risk factors (RR 10.6, 95 % CI 4.0-28.4) or had positive risk for all six risk factors including lipid measurements (RR 6.9, 95 % CI 1.2-41.4, P for difference 0.59).

Sensitivity analyses were conducted using study- and visit-specific z-scores. The C-statistics (95% CI) were 0.698 (0.667-0.731) for the non-laboratory measurements and 0.717 (0.685-0.748) for the lipid measurements (online supplemental figure 1). This difference was statistically significant (P=0.022). IDI and NRI-values (0.010 and 0.32, respectively, P always <

0.0002) showed small but significantly better discrimination for lipid based risk factors compared with non-laboratory risk factors (online supplemental table 3). The IPP reclassification of high cIMT probability based on adding LDL, triglycerides and HDL to the non-laboratory regression equation led to an improvement in prediction (online supplemental table 4). Cumulative incidence for high cIMT was 7.2 % in subjects whose risk was reclassified downward by adding lipids versus 11.2 % in subjects whose risk was reclassified upward with a difference of 4.0 % (P=0.0005).

Further analysis was done by using study specific risk factor z-scores to calculate whether replacing LDL-cholesterol with non-HDL-cholesterol would improve the prediction model. The results were essentially similar (LDL-cholesterol model: AUC [95% CI] 0.716 [0.685-0.748] versus non-HDL-cholesterol model: 0.717 [0.685-0748] P for difference 0.73). When triglycerides and HDL-cholesterol were replaced with triglyceride/HDL-cholesterol-ratio in the lipid model the prediction values for AUC and NRI for high adult cIMT were no longer statistically significant compared with the non-lab model: AUC (95 % CI) 0.709 (0.677-0.741), P-value 0.11; IDI 0.0046, P-value 0.01, NRI 0.067, P-value 0.067. Additional analyses were made using insulin as a marker of insulin resistance in childhood. Insulin data was available for 2,475 participants from YFS, BHS and IS cohorts. Prediction was not improved when insulin was included in the lipids model (Model 1) compared to lipids model without insulin (AUC, NRI, IDI P-value always > 0.11). Results for YFS, BHS and IS are presented in the online supplemental table 3. Although physical activity data were not available in all cohorts, a physical activity index was obtained at baseline in the YFS cohort (see online supplement). The results remained similar (data not shown) when this was added to separate analyses conducted in the YFS.

Discussion

The findings from four international cohorts examined in this study show that the risk of early atherosclerosis, represented by measurements of cIMT in young adults, could be predicted by non-laboratory measures (BMI, BP, smoking) of CVD risk factors in adolescence. The addition of blood lipids statistically improved models of prediction for pre-clinical atherosclerosis in adulthood.

In prior reports from these and other cohorts, it has been shown that childhood risk factors, and especially their clustering, are predictive of adult cIMT, as well as other non-invasive measures of preclinical atherosclerosis[1,17]. Moreover, findings from the BHS and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study have shown that CVD risk factors are strongly associated with early atherosclerotic lesions found in autopsies of youth dying from non-CVD related causes[7]. The findings in this study are consistent with these earlier observations showing the relation of early risk factor profiling to CVD pathophysiology.

In the NHANES I adult population (baseline age 25-74 years, N=14,407) Gaziano et al[3] examined whether a risk prediction method that did not require any laboratory tests could be as accurate as one requiring laboratory information. They observed that a model with non-laboratory-based risk factors predicted cardiovascular events as accurately as one that relied on laboratory-based values. The non-laboratory model included age, blood pressure, smoking, BMI, history of diabetes and history of blood pressure treatment, whereas in the laboratory based model BMI was replaced with total cholesterol levels. Consistent with these previous data, our findings from four cohort studies among youth aged 12 to 18 years (with no history of diabetes or treatment for blood pressure and hypercholesterolemia) were essentially similar concerning the association between adolescent risk factors and increased cIMT when the laboratory based

model substituted BMI for lipids – a similar approach used by Gaziano et al. In the present study, the results also showed statistically better discrimination for laboratory based risk factors over non-laboratory based risk factors when BMI was included in both models. While the addition of blood lipids statistically improved models of prediction for future pre-clinical atherosclerosis, the difference was modest with unclear clinical significance. Further studies are needed to clarify the predictive utility from measuring lipids at this age.

We have previously found in a pediatric setting by using pooled data from the YFS and the BHS, that high BMI alone was as good as and in some cases was superior to clustering of risk factors (high insulin, low-HDL-cholesterol, high triglycerides, high blood pressure and high BMI) in predicting high cIMT in adulthood[23]. Moreover, our previous analyses have shown an increase in CVD risk associated with childhood overweight or obesity and the tracking of adiposity between childhood and adulthood[24]. It was also stipulated that one measurement of BMI is more accurate than one measurement of the laboratory components. It is not known whether prediction using a lipid model could be improved by including lipid measurements obtained at repeated clinic visits over time[25]. Repeated lipid measurements were not available in the present study. These findings about the impact of lipids on future cIMT should be interpreted with caution given the biologic variability in lipid measurements.

Based on the present study the cumulative risk factor burden was quite similar between the non-laboratory and lipid models, suggesting that a risk assessment using only non-laboratory data is a useful alternative in the absence of information on lipids in predicting adult preclinical atherosclerosis. This would allow the immediate identification of youth at elevated risk who might benefit from therapeutic lifestyle intervention. Obvious benefits include avoiding the need to subject a child to a blood draw. Additional costs associated with lipid measurement in

childhood could include unnecessary worry, and time to interpret and follow-up on results. The revised pediatric guidelines recommend lipid screening both for identifying familiar hypercholesterolemia (FH), and for predicting atherosclerosis[2]. We acknowledge that our findings do not address the question of screening for FH in childhood, which is an important reason to measure lipid levels in childhood.

Although evidence suggesting that CVD has its origins in childhood is strong[1], there is no widely accepted childhood risk stratification system that uses risk factor data obtained from apparently healthy youths. From the PDAY data, a risk score has been developed estimating the probability for coronary artery lesions observed in autopsy, but it is only applicable for individuals aged 15-34 years[26,27]. After performing risk stratification, the main issue is how to intervene among high-risk children. In this sense, the results from the STRIP study show promise in that dietary intervention initiated in infancy has shown favorable effects on cardiovascular risk factors and arterial function[28]. In addition, among children with extreme cardiovascular risk such as in FH, statins have been shown to be effective in reducing the progression of preclinical atherosclerosis[29], though long-term efficacy has not been determined. Much variation exists in smoking across countries, regions, races, and social groups. During the past three decades, smoking prevalence among youth has fluctuated in unexpected ways that may have an impact on epidemiological studies. While major efforts have taken place to reduce smoking among adolescence, new trends such as other drug use have increased the use of tobacco among youths[30].

The main strength of this study is the use of pooled data on childhood risk factors and adult cIMT from four international longitudinal cohorts. However, the study has some potential limitations. First, because the study cohorts are comprised of young adults at follow-up, it was

not possible to study associations between risk factors and definite cardiovascular events. Instead, we have used cIMT as a surrogate end-point, but one that has been associated with future overt CVD. Second, study participants were predominantly Caucasian, and the results may not be generalizable to other ethnicities. Third, we were unable to consider pubertal stage or family history, both of, which have an influence on CVD risk factors, because data were not available for all cohorts. Waist circumference could not be used, because it was not collected at baseline in these cohorts. However, BMI, which is highly correlated to waist circumference, previously has been shown to give similar results as waist circumference in pediatric settings[31,32]. Finally, the analyses were based on adolescent risk factors, so the analyses are not necessarily applicable in younger children. However, we have previously shown that the association between childhood risk factors and adult preclinical atherosclerosis is weak or nonsignificant before the age of 11 years[33].

In summary, our data from four international cohort studies show that non-laboratory risk factors and lipids independently predict preclinical atherosclerosis in young adulthood. Although we found the predictive value of an approach that additionally considered lipids to be statistically superior to an approach that only considered non-laboratory factors, the clinical utility of lipid measurements remains uncertain given the modest improvements in risk prediction of preclinical atherosclerosis.

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Disclosures

None

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Table 1. Baseline characteristics in the four study cohorts

	Young Finns Study (1986)	Childhood Determinants of Adult Health Study (1985)	Bogalusa Heart Study (1984-1988)	The Insulin Study (2000-2008)	P-value*	All
N	2,079	272	436	106		2,893
Age, years	14.1±2.3	13.9±1.5	15.9±2.1	18.4±0.3	<0.0001	14.5±2.4
Sex (males %)	45	50	41	55	0.59	46
Systolic blood pressure, mmHg	114±11	112±13	110±10	111±9	<0.0001	113±11
Diastolic blood pressure, mmHg	66±10	66±12	57±11	60±13	<0.0001	65±11
Smoking, %	12	13	19	18	0.0002	14
BMI, kg/m^2	19.3±2.9	19.5±2.8	21.9±4.5	25.4±6.2	<0.0001	19.9±3.7
LDL cholesterol, mmol/L	3.21±0.81	2.68±0.70	2.46±0.75	2.30±0.66	<0.0001	3.01±0.84
HDL cholesterol, mmol/L	1.57±0.31	1.44±0.29	1.46±0.48	1.13±0.27	<0.0001	1.52±0.35
Triglycerides, mmol/L	0.80±0.36	0.72±0.31	0.86±0.40	1.09±0.58	<0.0001	0.81±0.38

* Age- and sex adjusted group comparisons among study cohorts
Data are mean ± SD, or proportions
Abbreviations: BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein

Table 2. Univariate relative risks for high cIMT in adulthood according to non-laboratory risk factors and lipids

Adolescent risk factor		RR	95%CI
Age (years)		1.10	1.04-1.17
Sex (male)		2.97	2.25-3.92
Blood pressure*	Normotensive	1.00	ref
	Prehypertensive	1.62	(1.21-2.18)
	Hypertensive	3.04	(2.08-4.43)
BMI†	Normal weight	1.00	ref
	Overweight	2.14	(1.47-3.13)
	Obese	4.26	(2.36-7.68)
Smoking	<1 cigarette/week	1.00	ref
	≥1 cigarette/week	1.57	1.12-2.18
LDL cholesterol	Normal (<2.85 mmol/L)	1.00	ref
	Borderline-high (≥2.85-3.36 mmol/L)	1.57	1.14-2.16
	High (≥3.37 mmol/L)	1.48	1.09-2.01
HDL cholesterol	Normal (>1.56 mmol/L)	1.00	ref
	Borderline-low (1.56-0.91 mmol/L)	1.67	1.26-2.20
	Low (<0.91 mmol/L)	2.34	1.25-4.39
Triglycerides	Normal (<1.02 mmol/L)	1.00	ref
	Borderline-high(≥1.02-1.46 mmol/L)	1.15	0.82-1.62
	High (≥1.46 mmol/L)	1.47	0.92-2.36

cIMT = carotid intima media thickness; high cIMT = study cohort specific ≥90th percentile
* age- and sex specific values defined according to the Fourth Report on High Blood Pressure in Children and Adolescents from the National High Blood Pressure Education Program[19]
† age- and sex specific values defined according to the Cole classification[18]
SI-unit conversion to mg/dL for lipids:
LDL: Normal <110mg/dL, Borderline High 110 - 129mg/dL, High ≥130mg/dL
Triglycerides: Normal < 90, Bordeline High 90 – 129mg/dL, High ≥130mg/dL
HDL: Normal >45mg/dL, Borderline Low 40 – 45mg/dL, Low < 40 mg/dL

Table 3. Multivariable relative risks for high cIMT in adulthood according to a non-laboratory model that includes only non-laboratory risk factors and a lipid model that also includes lipids measured in adolescence

	Adolescent risk factor		RR	95%CI
Non-laboratory				
	Age	Years	1.04	0.97-1.10
	Sex	Male	2.71	2.05-3.60
	Blood pressure*	Normotensive	1.00	ref
		Pre-hypertensive	1.45	1.07-1.98
		Hypertensive	2.12	1.41-3.19
	BMI†	Normal weight	1.00	ref
		Overweight	2.02	1.37-2.99
		Obese	3.69	1.99-6.83
	Smoking	<1 cigarette/week	1.00	ref
		≥1 cigarette/week	1.27	0.87-1.85
Lipid				
	Age	Years	1.04	0.97-1.11
	Sex	Male	2.70	2.03-2.59
	Blood pressure*	Normotensive	1.00	ref
		Pre-hypertensive	1.40	1.03-1.91
		Hypertensive	1.91	1.26-2.90
	BMI†	Normal weight	1.00	ref
		Overweight	1.98	1.36-2.90
		Obese	3.68	1.95-6.97
	Smoking	<1 cigarette/week	1.00	ref
		≥1 cigarette/week	1.28	0.87-1.86
	LDL cholesterol	Normal (<2.85 mmol/L)	1.00	ref
		Borderline-high (≥2.85-3.36 mmol/L)	1.60	1.15-2.22
		High (≥3.37 mmol/L)	1.55	1.12-2.14
	HDL cholesterol	Normal (>1.56 mmol/L)	1.00	ref
		Borderline-low (1.56-0.91 mmol/L)	1.36	1.01-1.83
		Low (<0.91 mmol/L)	1.36	0.66-2.77
	Triglycerides	Normal (<1.02 mmol/L)	1.00	ref

		Borderline-high(≥1.02-1.46 mmol/L)	0.92	0.63-1.33
		High (≥1.46 mmol/L)	0.92	0.54-1.57

Abbreviations: RR = relative risk, CI = confidence interval (Data are pooled, N = 2,893 for both non-lab and lab models); cIMT = carotid intima media thickness; high cIMT = study cohort specific ≥90th percentile

* age- and sex specific values defined according to the Fourth Report on High Blood Pressure in Children and Adolescents from the National High Blood Pressure Education Program[19]

† age- and sex specific values defined according to the Cole classification[18]

SI-unit conversion to mg/dL for lipids:

LDL: Normal <110mg/dL, Borderline-high 110 - 129mg/dL, High ≥130mg/dL

Triglycerides: Normal < 90, Bordeline-high 90 – 129mg/dL, High ≥130mg/dL

HDL: Normal >45mg/dL, Borderline-low 40 – 45mg/dL, Low < 40 mg/dL

Table 4. Performance and comparisons of adolescence non-laboratory model (non-laboratory risk factors) with lipid model (non-laboratory risk factors + lipids) in predicting adult high cIMT.

	H-Lχ2	AUC	95% CI	P-value for AUC difference	IDI	P-value for IDI	NRI	P-value for NRI
Non-laboratory	6.91	0.688	0.655-0.721	ref	ref	ref	ref	ref
Lipid (Model 1)	15.9	0.701	0.669-0.733	0.038	0.0045	0.007	0.19	0.003
Lipid (Model 2)	3.94	0.691	0.659-0.723	0.39	0.007	0.10	0.002	0.97

Lipid model = non-laboratory risk factors + lipids
Model 1 including all risk factors (age, sex, blood pressure, BMI, triglycerides, LDL- and HDL-cholesterol).
Model 2 BMI replaced with lipids (age, sex, blood pressure, triglycerides, LDL- and HDL-cholesterol)
Abbreviations: H-Lχ2,=Hosmer-Lemeshow χ2 statistics, AUC=Area under the curve, CI=confidence intervals, IDI=Integrated discrimination improvement measurement, NRI=Net reclassification improvement measurement. Models are adjusted for study year and race.
Risk factor cutoff values according to international criteria[18-20]. cIMT = carotid intima media thickness; high cIMT = study cohort specific ≥90th percentile

Table 5. Improvement in Prediction Probability for non-laboratory- and lipid models

	Reclassification down: % events (n/N)	Reclassification up: % events (n/N)	Relative risk	P categorical reclassification	P continuously graded reclassification
High cIMT	6.35 (90/1418)	9.70 (105/1082)	1.43	0.0021	0.0078

P based on 2-sample z test for proportions comparing cumulative events rates for those reclassified up versus reclassified down

P for graded reclassification (Poisson regression of outcome on reclassification probability adjusted for continuous base risk)

cIMT = carotid intima-media thickness

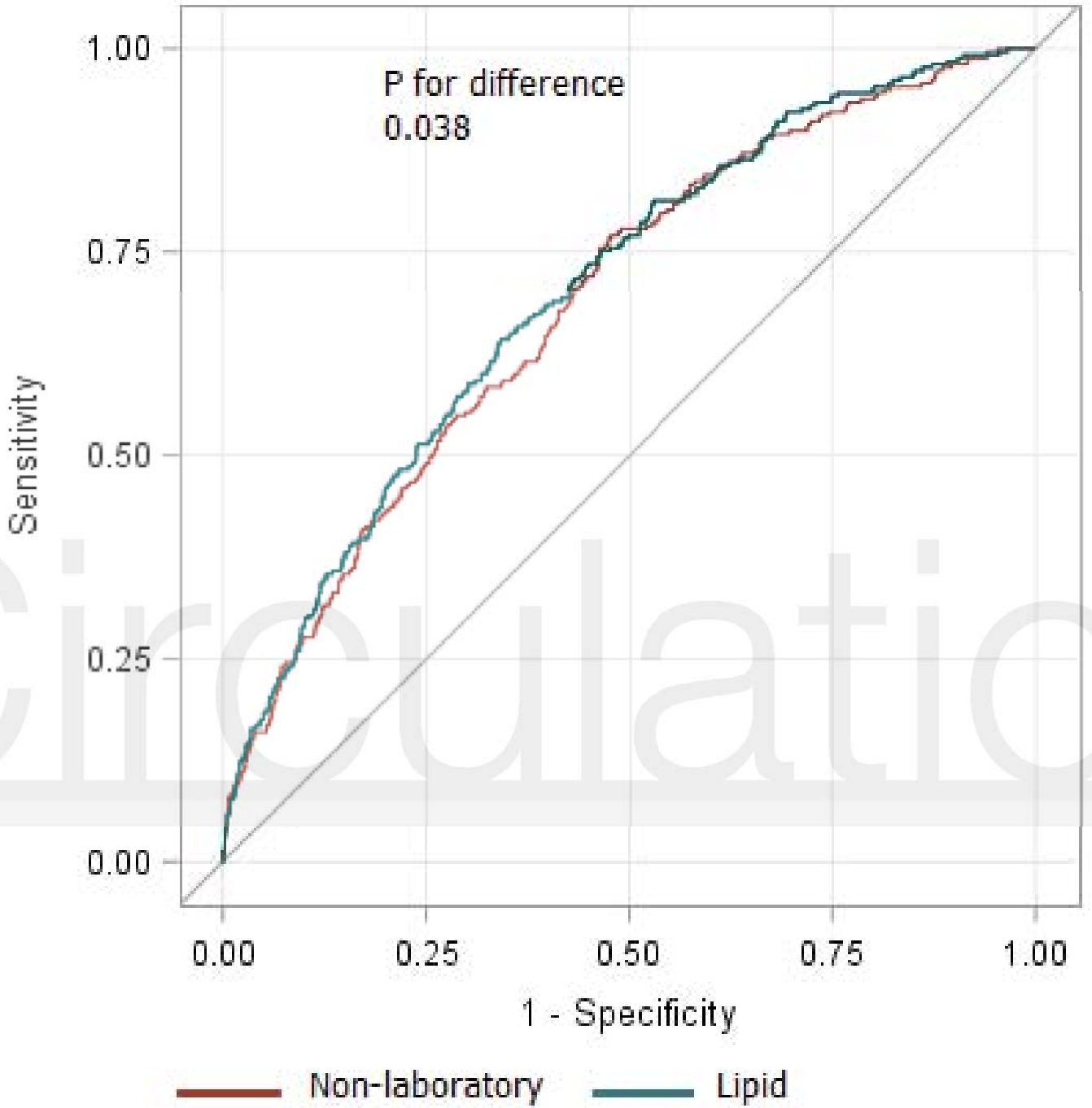
Figure Legends

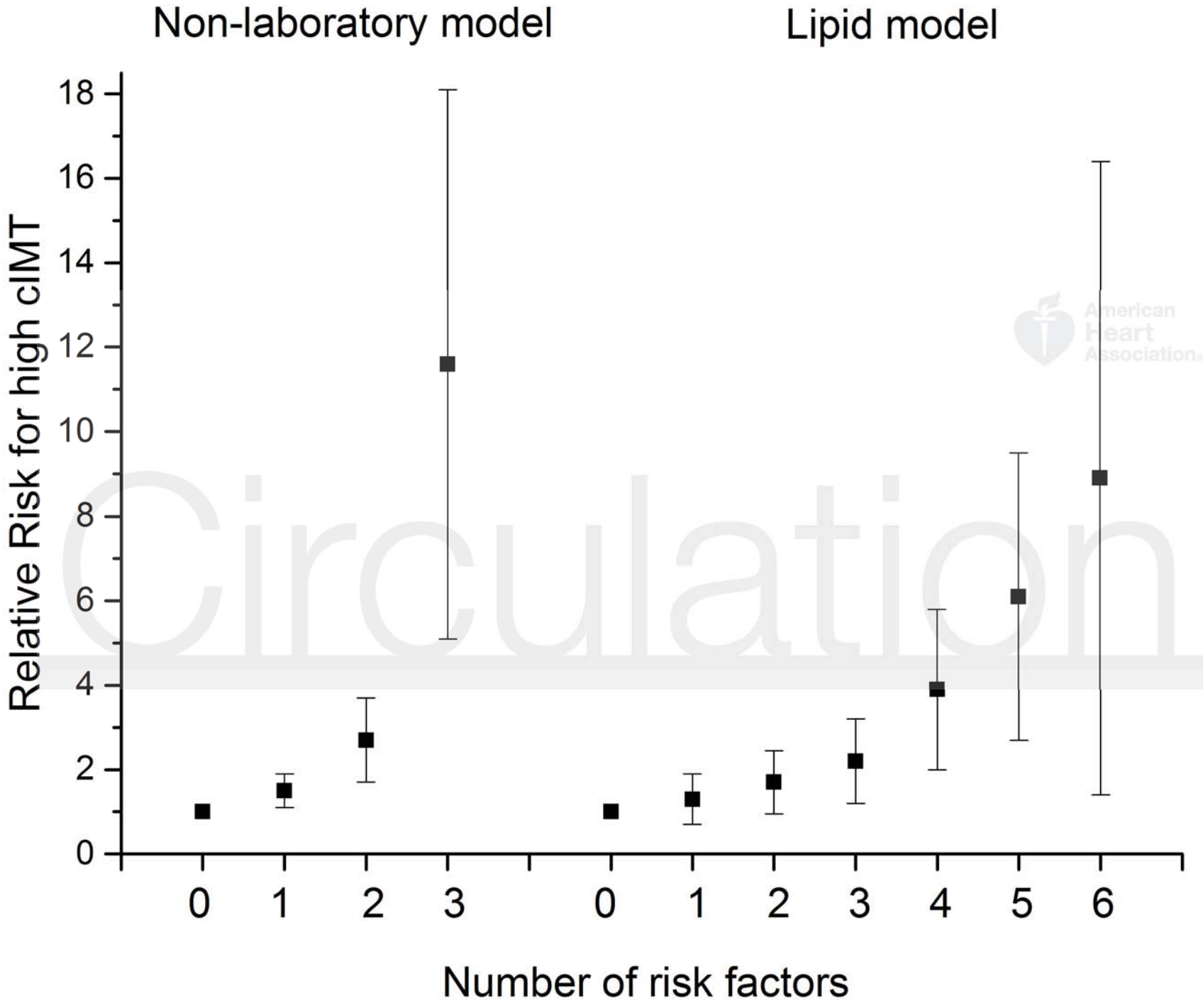
Figure 1. Receiver operating characteristic (ROC) curves comparing non-laboratory (age, sex, blood pressure, BMI, smoking) and lipid (non-laboratory plus lipids) cardiovascular risk factors in adolescents for prediction of adult carotid IMT ≥90th percentile.

C-statistic (95% CI): non-laboratory = 0.688 (0.655-0.721); laboratory = 0.701 (0.669-0.733). P for difference 0.038. Lipid model = non-laboratory risk factors + lipids, cIMT = carotid intima media thickness

Figure 2. The risk relative to participant with no risk factors (relative risk) for high cIMT according to the number of risk factors stratified by non-laboratory- and lipid (non-laboratory + lipids) models.

If a risk factor was above (BMI, blood pressure, LDL-cholesterol, triglycerides) or below (HDL-cholesterol) normal cut-points derived from the recent recommendations from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents[18-20] or a participant reported smoking, the risk factor was considered positive. Lipid model = non-laboratory risk factors + lipids, cIMT = carotid intima media thickness





Impact of Lipid Measurements in Youth in Addition to Conventional Clinic-Based Risk Factors on Predicting Preclinical Atherosclerosis in Adulthood: The International Childhood Cardiovascular Cohort (i3C) Consortium

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SUPPLEMENTAL MATERIAL

Impact of lipid measurements in youth in addition to conventional clinic-based risk factors on predicting preclinical atherosclerosis in adulthood. The International Childhood Cardiovascular Cohort (i3C) Consortium

Methods

Smoking questionnaires

In BHS subjects were asked at baseline (ages 8 to 17y) and follow-up about smoking. participants were categorized as: 1) currently smokes at least one cigarette a week (≥1/week), 2) currently experimenting with cigarettes (fewer than one cigarette per week) (<1/week), 3) used to smoke at least one cigarette a week but no longer smokes cigarettes (former) 4) at one time was experimenting (<1/week) with cigarettes but quit smoking (former experimenter) 5) never experimented with cigarettes (never). In YFS at baseline (those aged 12 years or older) and follow-up, participants were categorized: 1) once per day or more often (≥1/day), 2) at least once per week, but not daily (≥1/week), 3) less than once per week (<1/week), 4) stopped smoking or do not smoke at present (former), 5) never smoked (never). In CDAH at baseline, participants aged 9 years and older were asked a series of questions on smoking behavior that included the following question: 'How long have you been smoking regularly? (regularly means 1 or more times a week)' and categorized as follows 1) 'I don't smoke'; 2) 'just started'; 3) '1 to 6 months'; 4) '7 months to 1 year'; 5) '1 to 2 years'; 6)'2 to 4 years'; 7)'more than 4 years'. In IS, subjects categorized whether subject was 1) Smoking regularly, 2) Not smoking regularly. These responses were collapsed into a binomial categorical variable indicating: 1) regular smoking or at least once per week (≥1/week), i.e. those that indicated any of options 2-7. 2) No smoking or less than once per week (<1/week), i.e. those that indicated 'I don't smoke'.

Physical activity questionnaires

The participants were asked to report their habitual leisure-time physical activity intensity, frequency, and duration[1]. A metabolic equivalent (MET) index for leisure-time physical activity (later called "MET-index") was calculated from the product of intensity×frequency×duration (MET h/wk). The coefficients for the intensity of physical

activity were estimated from the existing tables. One MET is the consumption of 1 kcal of a person per weight kilogram per hour in rest. The MET-index ranged between 0 and 52 MET h/wk in 1986.

Insulin samples

Additional analyses were made using insulin as a marker of insulin resistance in childhood. Insulin data was available for 2,475 participants from YFS, BHS and IS cohorts. In YFS serum insulin concentrations were measured by microparticle enzyme immunoassay kit (CV 2.1%) (Abbott Laboratories, Diagnostic Division, Dainabot). In BHS, a commercial radioimmunoassay kit was used to measure plasma insulin levels (Phadebas, Pharmacia Diagnostics, Piscataway, NJ). In IS the insulin samples were determined with a radioimmunoassay kit (Equate RIA, Binax Corp, Portland, ME, USA).

RESULTS

Non-laboratory- vs Laboratory (i.e. Non-laboratory with additional lipid measurements) based risk assessment in predicting high cIMT in adulthood

Supplemental table 1 shows the results for a univariable model assessing risk ratios for high cIMT (≥90th percentile) in adulthood according to individual risk factors measured between 12 to 18 years of age. Age, sex, systolic blood pressure, BMI, smoking, LDL- and HDL-cholesterol in adolescence were associated with high adult cIMT (P always < 0.05), but not triglycerides (P=0.17). Multivariable analyses (Supplemental table 2) assessing youth non-laboratory based risk factors and adult high cIMT showed significant associations for adolescent sex, systolic blood pressure and BMI, but not smoking. When lipids were introduced into the model, the association was significant for sex, systolic blood pressure, BMI, LDL- and HDL-cholesterol. Age, smoking and triglycerides were not independent predictors of high cIMT in the final model including all lipid measurements.

Supplemental figure 1 shows the results for receiver operating characteristic (ROC) curves for non-laboratory-based (age, sex, systolic blood pressure, BMI, smoking) and lipid-based (non-laboratory plus lipids) cardiovascular risk factors in adolescence for prediction of adult cIMT ≥90th percentile. The C-statistic (95% CI) for the non-laboratory measurements was significantly lower 0.698 (0.667-0.731) compared with the model that included the laboratory measurements 0.717 (0.685-0.748).

When study cohorts were analyzed separately, no significant differences between models were observed: for YFS the non-laboratory based AUC was 0.717 (0.682-0.753) and lipid based AUC 0.725 (0.689-0.760), P for difference 0.22. For CDAH the non-laboratory based

AUC was 0.605 (0.497-0.712) and lipid based AUC 0.691 (0.550-0.832), P for difference 0.30. For BHS the non-laboratory based AUC was 0.738 (0.657-0.820) and lipid based AUC 0.755 (0.675-0.834), P for difference 0.21. For IS the non-laboratory based AUC 0.757 (0.531-0.983) and lipid based AUC 0.903 (0.789-1.000), P for difference 0.09.

Supplemental table 3 provides data that compares the utility of lipid-based risk assessment with non-laboratory risk assessment in predicting adult high cIMT. The data for the non-laboratory based model (age, sex, systolic blood pressure, BMI, smoking) and lipid-based model (non-laboratory risk factors plus triglycerides, LDL and HDL cholesterol) in terms of AUC, IDI and NRI-values showed significantly better discrimination with the lipid based model (Model 1). Model 2 shows the results when a comparison was made between non-laboratory and lipid-based models where BMI is removed and replaced with lipids in the lipid-based model. AUCs were similar between the models predicting high cIMT (P for difference 0.15). In addition, no significantly improved IDI or NRI (0.004 and 0.063, P for difference always > 0.32) was observed with the lipid-based model over the non-laboratory based model (Supplemental table 3). Goodness-of-fit indicated by the Hosmer-Lemeshow $\chi 2$ was acceptable for all models examined. The IPP reclassification of high cIMT probability based on adding lipids to the base regression equation led to an improvement in prediction (Supplemental table 4). Cumulative incidence for high cIMT was 7.19 % in subjects whose risk was reclassified downward by adding lipids versus 11.20 % in subjects whose risk was reclassified upward with a difference of 4.01 % (P=0.0005) which is added discrimination of 45 % of the average risk for everyone. The Poisson model showed similar results but a lower reclassification percentages compared to the linear model (6.74 % in subjects whose risk was reclassified downward versus 9.91 % in subjects whose risk was reclassified upward, P for difference 0.0026).

SUPPLEMENTAL TABLE 1. Univariable relative risks for high cIMT in adulthood according to non-laboratory and laboratory risk factors measured in adolescence

Risk factor	RR	95% CI	P-value
Age (years)	1.10	1.04-1.17	0.0009
Sex (male)	2.97	2.25-3.92	<0.0001
Systolic blood pressure*	1.54	1.36-1.74	<0.0001
Body mass index*	1.51	1.35-1.68	<0.0001
Smoking (yes)	1.57	1.13-2.18	0.0076
LDL-cholesterol*	1.24	1.10-1.40	0.0003
HDL-cholesterol*	0.71	0.62-0.82	<0.0001
Triglycerides*	1.10	0.97-1.25	0.13

Abbreviations: RR = risk ratio; CI = confidence interval; cIMT = carotid intima media thickness cIMT = carotid intima media thickness

high cIMT = study cohort and -year specific $\geq 90^{th}$ percentile

*Study cohort and year specific z-score

SUPPLEMENTAL TABLE 2. Multivariable relative risks for high cIMT in adulthood according to non-laboratory and laboratory risk factors measured in adolescence

	Adolescent risk factor	RR	95 % CI	P-value
Non-laboratory				
	Age (years)	1.06	0.99-1.12	0.09
	Sex (male)	2.88	2.16-3.84	<0.0001
	Blood pressure*	1.25	1.08-1.43	0.0015
	BMI*	1.36	1.19-1.53	<0.0001
	Smoking (yes)	1.15	0.81-1.63	0.44
Lipid				
	Age (years)	1.06	0.99-1.12	0.92
	Sex (male)	2.72	2.10-3.78	<0.0001
	Blood pressure*	1.25	1.08-1.43	0.0016
	BMI*	1.33	1.16-1.52	<0.0001
	Smoking (yes)	1.17	0.82-1.67	0.39
	LDL cholesterol*	1.31	1.15-1.49	<0.0001
	HDL cholesterol*	0.82	0.70-0.96	0.012
	Triglycerides*	0.87	0.74-1.01	0.064

Abbreviations: RR = relative risk, CI = confidence interval; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein, cIMT = carotid intima media

thickness; cIMT = carotid intima media thickness; high cIMT = study cohort and -year specific ≥90^{th} percentile

*Study specific z-score

SUPPLEMENTAL TABLE 3, Model performance and comparisons of youth non-laboratory risk factors with laboratory risk factors in predicting adult high cIMT (study cohort and -year specific).

	H-Lχ2	AUC	95% CI	P-value for AUC difference	IDI	P-value for IDI	NRI	P-value for NRI
Non-laboratory	6.64	0.698	0.667-0.731	ref	ref	ref	ref	ref
Lipid (model 1)	9.14	0.717	0.685-0.748	0.022	0.010	0.0002	0.320	<0.0001
Lipid (Model 2)	6.79	0.707	0.675-0.739	0.15	0.004	0.51	0.063	0.32
Lipid (Model 1) + Insulin*	8.70	0.722	0.690-0.755)	0.017	0.012	<0.0001	0.392	<0.0001

Abbreviations: H-Lχ2 = Hosmer-Lemeshow χ2 statistics, AUC = Area under the curve, CI = confidence intervals, IDI = Integrated discrimination improvement measurement, NRI = Net reclassification improvement measurement. cIMT = carotid intima media thickness.

Model 1 including all risk factors (age, sex, systolic blood pressure, BMI, triglycerides, LDL- and HDL-cholesterol).

Model 2 BMI replaced with lipids

*Analysis included only participants from YFS, BHS and IS studies who had insulin data available in adolescence (N=2,475).

SUPPLEMENTAL TABLE 4, Improvement in Prediction Probability for non-laboratory- and lipid model

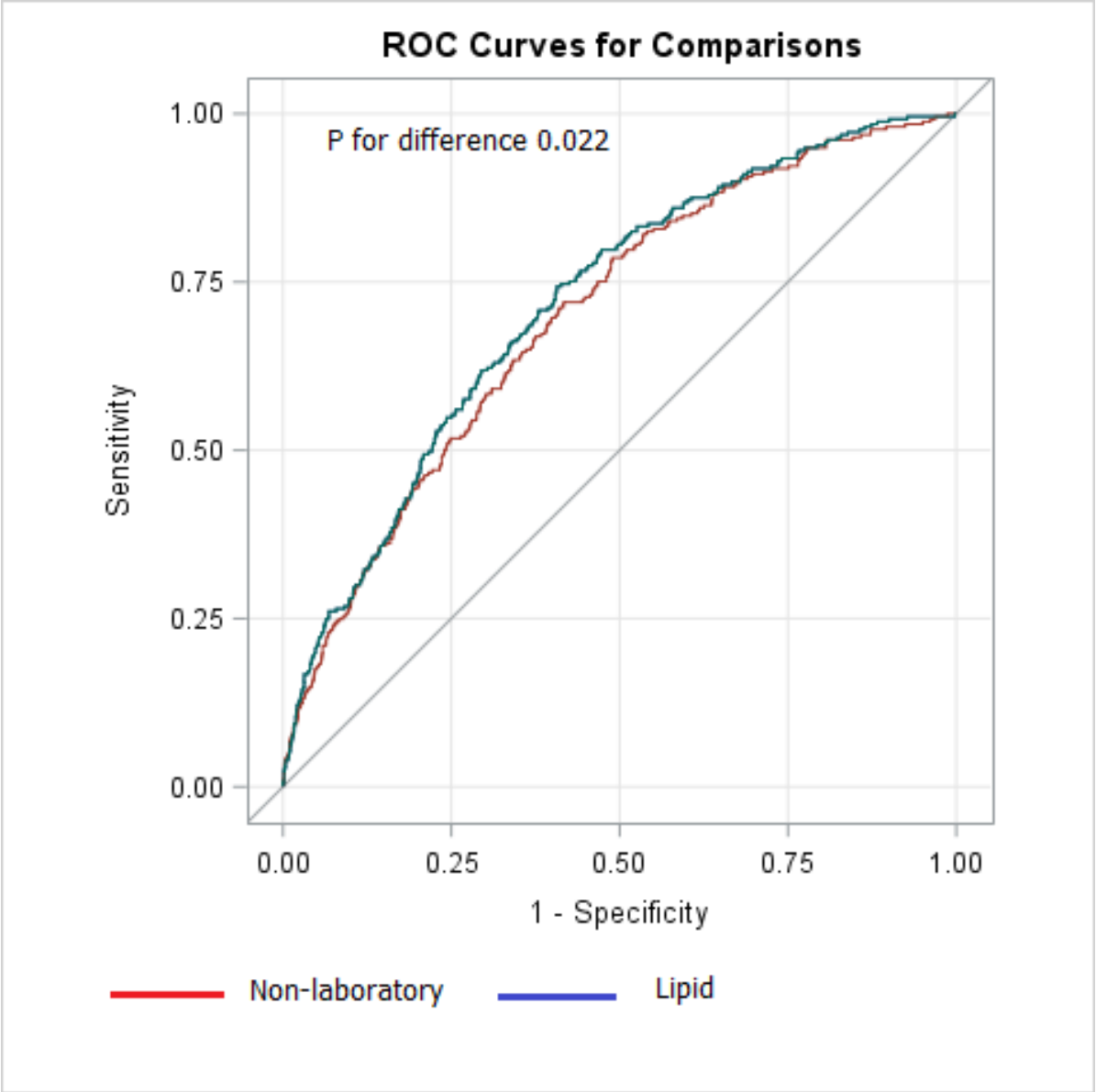
	Reclassification down: % events (n/N)	Reclassification up: % events (n/N)	Relative risk	P categorical reclassification	P continuously graded Reclassification
High cIMT	7.19 (115/1600)	11.20 (133/1187)	1.45	0.0005	0.0026

P based on 2-sample z test for proportions comparing cumulative events rates for those reclassified up versus reclassified down

P for graded reclassification (Poisson regression of outcome on reclassification probability adjusted for continuous base risk)

cIMT = carotid intima media thickness

SUPPLEMENTAL FIGURE 1.



Receiver operating characteristic (ROC) curves for non-laboratory-based (age, sex, blood pressure, BMI, smoking) and laboratory-based (non-lab plus lipids) cardiovascular risk factors in adolescence for prediction of adult carotid IMT ≥90th percentile. C-statistic (95% CI): non-laboratory = 0.698 (0.667-0.731); laboratory = 0.717 (0.685-0.748)

References

1. Raitakari OT, Porkka KV, Taimela S, Telama R, Rasanen L, Viikari JS. Effects of persistent physical activity and inactivity on coronary risk factors in children and young adults. The Cardiovascular Risk in Young Finns Study. *Am J Epidemiol.* 1994; 140: 195-205.