

## Non-HDL Cholesterol Levels in Childhood and Carotid Intima-Media Thickness in Adulthood

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**Table of Contents Summary:** We examined childhood non-HDL-C status predicts high common carotid artery intima-media thickness (cIMT) in adulthood.

**What's known on this subject:** Elevated non-high-density lipoprotein cholesterol (non-HDL-C) levels are used to identify children at increased cardiovascular risk but the utility of non-HDL-C in childhood to predict atherosclerosis is unclear.

**What this study adds:** Non-HDL-C levels associate with future risk of preclinical atherosclerosis from the age of 15 years, suggesting a later age for the initial universal lipid screening amongst the pediatric population than is currently recommended in the NHLBI's Expert Panel guidelines.

## **Contributors statements**

Drs Juonala, Wu and Magnussen conceptualized and designed the study, analyzed the data, drafted the initial manuscript, and reviewed and revised the manuscript.

Drs Sinaiko, Woo, Urbina, Jacobs, Steinberger, Prineas, Burns, Bazzano, Venn, Viikari, Hutri-Kähönen, Daniels, Dwyer and Raitakari designed and executed data collection, collected data, and critically reviewed and revised the manuscript for important intellectual content.

Drs Koskinen, Sabin and Burgner conceptualized and designed the study, interpreted the data, and critically reviewed and revised the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

## ABSTRACT

**Background:** Elevated non-high-density lipoprotein cholesterol (non-HDL-C) levels are used to identify children at increased cardiovascular risk but the utility of non-HDL-C in childhood to predict atherosclerosis is unclear. We examined whether the National Heart, Lung, and Blood Institute (NHLBI) classification of youth non-HDL-C status predicts high common carotid artery intima-media thickness (cIMT) in adulthood.

**Methods:** We analyzed data from four prospective cohorts among 4,582 children aged 3-19 years who were re-measured as adults (mean follow-up of 26 years). Non-HDL-C status in youth and adulthood was classified according to cut-points of the NHLBI and the National Cholesterol Education Program Adult Treatment Panel III. High cIMT in adulthood was defined as at or above the study visit-, age-, sex-, race-, and cohort-specific 90th percentile of IMT.

**Results:** In a log-binomial regression analysis adjusted with age at baseline, sex, cohort, length of follow-up, baseline body mass index and systolic blood pressure, children with dyslipidemic non-HDL-C were at increased risk of high cIMT in adulthood (relative risk (RR), 1.29; 95% confidence interval [CI], 1.07-1.55). Compared to the persistent normal group, the persistent dyslipidemia group (1.80[1.37-2.37]) and incident dyslipidemia (normal to dyslipidemia) groups (1.45[1.07-1.96]) had increased risk of high cIMT in adulthood, but the risk was attenuated for the resolution (dyslipidemia to normal) group (1.17[0.97-1.41]).

**Conclusion:** Dyslipidemic non-HDL-C levels predict youth at risk of developing high cIMT in adulthood. Those who resolve their non-HDL-C dyslipidemia by adulthood have normalized risk of developing high cIMT in adulthood.

## INTRODUCTION

Non-high-density lipoprotein cholesterol (non-HDL-C) is considered a simpler and more effective screening tool of atherosclerotic cardiovascular disease risk than low-density lipoprotein cholesterol (LDL-C)<sup>1, 2</sup>. Estimated LDL-C does not include all classes of atherogenic lipoproteins<sup>3</sup>, may underestimate those with low levels<sup>4</sup>, and requires overnight fasting<sup>2</sup>. Consequently, non-HDL-C is increasingly used<sup>5</sup> and has been specified as a secondary therapy target among patients with the metabolic syndrome or diabetes<sup>6</sup>.

We have previously reported that elevated LDL-C levels in children and adolescents (youth) were associated with high carotid intima-media thickness (cIMT), a marker of preclinical atherosclerosis, in adulthood<sup>7</sup>. For total cholesterol, our previous analyses suggested that measures from those aged  $\geq 12$  years, but not in younger children, were associated with adult cIMT<sup>8</sup>. The case for non-HDL-C measurement in youth is further strengthened by data showing youth non-HDL-C is a better predictor of adult dyslipidemia<sup>9</sup>, non-lipid risk factors<sup>9</sup>, and cIMT<sup>10</sup> than LDL-C. Moreover, non-HDL-C is independently associated with obesity indices<sup>11, 12</sup> and might provide a more sensitive measure of dyslipidemia than LDL-C amongst those with overweight or obesity<sup>13</sup>. Recognizing the potential value of non-HDL-C measurement, the 2011 National Heart, Lung, and Blood Institute (NHLBI) expert panel recommended universal (population-wide) screening of non-fasting non-HDL-C levels first at age 9-11 years and again at age 18-21 years to identify youth with dyslipidemia at risk for accelerated atherosclerotic disease<sup>14</sup>. In contrast to previous guidelines on lipid screening<sup>15, 16</sup>, the NHLBI guidelines were the first to recommend universal vs. selected screening for lipid disorders and to incorporate cut-offs for non-HDL-C levels, derived from population-based data in the Bogalusa Heart Study<sup>12</sup>. However, these data were cross-sectional and it is unknown whether the NHLBI cut-offs for non-HDL-C predict future preclinical atherosclerosis.

Using data from four population-based prospective cohorts beginning in youth with follow-up into adulthood, we examined if the NHLBI classification of youth non-HDL-C status is associated with adult cIMT. We also compared associations for LDL-C and examined whether resolution of elevated youth non-HDL-C status by adulthood reduces the risk of developing high cIMT.

## **METHODS**

### **Study sample**

The study sample was drawn from four prospective cohorts in the i3C Consortium<sup>17</sup>. These were the Bogalusa Heart Study (Louisiana, United States), the Insulin Study (Minneapolis, United States), the Cardiovascular Risk in Young Finns Study (Finland), and the Childhood Determinants of Adult Health (CDAH) Study (Australia). Study characteristics and methods have been previously described<sup>18-23</sup>. Although loss to follow-up varied by cohort, previous analyses have suggested the representativeness of the cohorts has largely been maintained<sup>17</sup>. In total, 4,582 participants with non-HDL-C data from their first study visit in youth when aged 3-19 years and longitudinal ultrasound data from adulthood when aged 19-51 years were included. Local ethics committees reviewed and approved the individual cohort studies that we analyzed, and participants in those studies (or their legal guardians) provided written informed consent. The present analysis conformed to the Declaration of Helsinki.

### **Risk factor assessment**

In the Young Finns Study 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. Serum HDL-C was measured by the dextran sulfate 500 000 method. In CDAH, serum total cholesterol and triglycerides were determined according to the Lipid Research Clinics Program<sup>24</sup>, and HDL-C was analyzed after precipitation of apolipoprotein B-containing lipoproteins with heparin-manganese<sup>25</sup>. In the Bogalusa Heart Study, HDL-C and triglycerides were measured using chemical procedures with a Technicon Auto Analyzer II (Technicon Instrument Corp), according to the Lipid Research Clinics Program<sup>24, 26</sup>. Commencing after baseline, these variables were determined by enzymatic procedures using

the Abbott VP instrument (Abbott Laboratories)<sup>27</sup>. Serum concentrations of LDL-C and HDL-C were analyzed by a combination of heparin-calcium precipitation and agar-agarose gel electrophoresis procedures. In the Insulin Study, serum lipids were analyzed in the University of Minnesota laboratory with a Cobas FARA<sup>28</sup>. HDL-C 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. For all cohorts, non-HDL-C was calculated as total cholesterol–HDL-C, and LDL-C was calculated using the Friedewald formula<sup>29</sup>. The coefficient of variation for within-assay precision in the Young Finns Study was 2.2 % for total cholesterol, 2.3 % for HDL-C, and 3.8 % for serum triglycerides. Both of the 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-C concentrations. Height and weight were measured and used to calculate body mass index (BMI) as  $\text{weight (kg)} / (\text{height (m)})^2$ . Cole's international BMI cut-offs<sup>30</sup> were used to denote weight status. Systolic blood pressure at baseline was measured using a standard mercury sphygmomanometer<sup>31</sup>. Youth smoking habits were assessed by questionnaire. Those who had smoked  $\geq 1$  cigarette/day in youth (<20 years old) were considered smokers.

### **Ultrasound measurements**

B-mode ultrasound studies of the left common carotid artery were performed at follow-up examinations using standardized protocols in each study. Details of the ultrasound data, protocols and reproducibility have been described elsewhere<sup>7, 32</sup>.

### **Exposure and outcome definitions**



In youth, non-HDL-C status was defined as normal  $<3.10$  mmol/l ( $<120$  mg/dL), elevated  $3.10$ - $<3.75$  mmol/l ( $120$ - $<145$  mg/dL), and dyslipidemia  $\geq 3.75$  mmol/l ( $\geq 145$  mg/dL), and LDL-C status as normal  $<2.85$  mmol/l ( $<110$  mg/dL), elevated  $2.85$ - $<3.36$  mmol/l ( $110$ - $<130$  mg/dL), and dyslipidemia  $\geq 3.36$  mmol/l ( $\geq 130$  mg/dL) according to cut-points from the NHLBI expert panel<sup>14</sup>. Non-HDL-C status in adulthood was defined as normal  $<4.91$  mmol/l ( $<190$  mg/dl) and dyslipidemia  $\geq 4.91$  mmol/l ( $\geq 190$  mg/dL), and for LDL-C was defined as normal  $<4.14$  mmol/l ( $<160$  mg/dL) and dyslipidemia  $\geq 4.14$  mmol/l ( $\geq 160$  mg/dL). Change in non-HDL-C and LDL-C status between youth and adulthood was defined as: persistent dyslipidemia (dyslipidemia at both time-points), incident dyslipidemia (normal to dyslipidemia), resolution (dyslipidemia to normal) and persistent normal (normal at both time-points). The latest available measurement of cIMT was used and high cIMT in adulthood was defined as at or above the follow-up year-, age-, sex-, race-, and cohort-specific 90th percentile. As there was a small number of participants in some categories after stratification by age, sex, race, cohort and follow-up years (where the proportion of high-risk cIMT ranged between 10-20%), the combined average rate for the pooled data was higher than the expected 10%.

## **Statistical analysis**

Univariable and/or multivariable modified Poisson regression models (using a robust error variance) were used to estimate the relative risk (RR) and 95% confidence intervals (CI) for the association of youth lipids and changes in their status between youth and adulthood with adult risk of having high carotid IMT. As weight status is thought to influence the predictive utility of non-HDL-C vs. LDL-C, we performed a sensitivity analysis stratified by Cole's BMI weight categories<sup>30</sup>. There were significant interactions between youth non-HDL-C and LDL-C status with cohort and youth age but not sex. Therefore, we also conducted

multivariable modified Poisson regression models for associations between youth non-HDL-C and LDL-C status and adult risk of high IMT stratified by cohort, and youth age groups (3-8, 9-11, 12-14, 15-17 and 18+ years). Youth age groups were based on current risk screening age windows used by the NHLBI where universal screening occurs from age 9-11 years and again at 18+ years<sup>14</sup>. All multivariable analyses were adjusted for age, BMI and systolic blood pressure at baseline, sex, cohort, and length of follow-up. Logistic regression models (all covariates adjusted above were included in the model) were used to obtain area under receiver-operating curve (AUC) values to estimate and compare the predictive utility of youth non-HDL-C and LDL-C on adult risk of having high IMT. All analyses were re-run after additional adjustment for youth smoking or exclusion of those individuals having lipid-lowering medication (n=155, all in adulthood; 112 from BHS, 2 from CDAH and 41 from YFS) and risk ratios remained essentially similar in these analyses. As a sensitivity analysis funnel plots were generated for the association of non-HDL-C or LDL-C status in youth with carotid artery IMT  $\geq$ 90th percentile in adulthood. Analyses were performed in Stata version 15.1 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

## RESULTS

Characteristics are shown in Supplemental Table 1. Youth characteristics among those who had elevated non-HDL-C or LDL-C are presented in Table 1. Of those with elevated youth non-HDL-C levels, 2335 (94%) also had elevated youth LDL-C levels. Moreover, 150 participants had elevated youth non-HDL-C but not elevated LDL-C levels and 70 had elevated youth LDL-C but not elevated non-HDL-C levels. 47 % of those with elevated youth non-HDL-C levels had elevated levels as adults compared with 57 % of those with elevated youth LDL-C levels having elevated levels as adults (Table 1).

Pooled RR and their 95% CIs for high adult cIMT according to non-HDL-C and LDL-C status in youth are shown in Table 2. Compared to those classified as not having dyslipidemia, youth with non-HDL-C dyslipidemia or LDL-C dyslipidemia were at increased risk of high cIMT in adulthood. Additional adjustment for youth BMI and systolic blood pressure (Model 2, Table 2) did not appreciably change the effect estimates. The AUC was comparable between non-HDL-C and LDL-C models, with AUCs of 0.622 and 0.620 respectively,  $P > 0.5$ . The AUC was similar between non-HDL-C and LDL-C when stratified by BMI status (normal weight, non-HDL-C AUC of 0.630 vs. LDL-C AUC of 0.628,  $P > 0.5$ ; overweight/obese, non-HDL-C AUC of 0.552 vs. LDL-C AUC of 0.549,  $P > 0.5$ ). A sensitivity analysis utilizing funnel plots showed an asymmetry in the scatter of small studies with more studies showing a lower-magnitude association between youth non-HDL-C or LDL-C with adult risk of cIMT (Supplemental Figure 1). In an additional pooled analysis based on 3 smaller cohorts (ie. excluding the Young Finns data) youth non-HDL-C dyslipidemia or LDL-C dyslipidemia were not associated with the risk of high cIMT in adulthood (Supplemental Table 2).

Age-stratified results for the association between youth non-HDL-C and LDL-C status with adult high cIMT are shown in Table 3. Those with dyslipidemia aged 15-17 years had

significantly increased risk of adult high cIMT, as did those aged 18 years or over with elevated non-HDL-C or LDL-C status.

Table 4 shows results for adult high cIMT by youth non-HDL-C and LDL-C status, stratified by cohort. Within each cohort, associations were similar for both elevated and dyslipidemia non-HDL-C and LDL-C classifications. Between each cohort, there was large heterogeneity in effect estimates for elevated and dyslipidemia status in both non-HDL-C and LDL-C, with only participants in the Young Finns Study showing a consistent and graded increase in risk for adult high cIMT based on youth non-HDL-C or LDL-C status.

Table 5 shows the pooled RR and their 95% CIs for high adult cIMT according to youth and adult non-HDL-C and LDL-C status. Compared with the persistent normal non-HDL-C group, those with persistent or incident non-HDL-C dyslipidemia had increased risk of high cIMT in adulthood; increased but weaker associations were observed for resolution and incident non-HDL-C dyslipidemia groups. Based on LDL-C classification in youth and adulthood, only those with persistent dyslipidemia had significantly increased risk of high adult cIMT compared with the normal LDL group (Table 5). We observed very similar risk estimates after further adjustment for youth BMI and systolic blood pressure (Model 2, Table 5).

## DISCUSSION

These longitudinal data suggest that youth non-HDL-C levels are associated with high cIMT, in adulthood. In age-stratified analyses, this relationship was observed for non-HDL-C measurements performed from the age of 15 years. The predictive utility for high adult cIMT was similar using either LDL-C or non-HDL-C.

We have previously reported that elevated youth LDL-C levels<sup>7</sup> and total cholesterol<sup>8</sup> were associated with high cIMT in adulthood. For LDL-C we observed that only persistent dyslipidemia was related to higher cIMT<sup>7</sup>. For total cholesterol, our prior analyses suggested that the measures in those aged  $\geq 12$  years, but not in younger children, were associated with high adult cIMT<sup>32</sup>. The present data for non-HDL-C are in line with these observations but provide important additional information that non-HDL-C levels in youth, especially among those aged  $\geq 15$  years, are predictive of cIMT. In analyses taking into account both youth and adult non-HDL-C levels, those with elevated adult non-HDL-C levels had high cIMT independent of their youth non-HDL-C status. Among those individuals with elevated non-HDL-C in youth but not in adulthood, the increased risk for developing high cIMT was partly attenuated (RR 1.17, 95% CI 0.97-1.41). In cohort-specific analyses our findings were somewhat different, particularly for the US cohorts. Only in the Young Finns Study we observed consistent relations between youth lipids with adult carotid IMT, whereas pooled analyses restricted on 3 smaller cohorts showed no associations. Possible explanations for this are complex and may include the larger sample and case number size (as suggested by our funnel plots), longer follow-up time, higher childhood lipid levels in the Young Finns Study (highest in the world in 1970s<sup>33</sup>) providing larger lifetime lipid exposure, and differences in lipid measurement and IMT methodology across the cohorts.

The 2011 NHLBI Expert Panel Guidelines<sup>14</sup> were the first to suggest universal lipid screening in youth, initially at age of 9-11 years, and again at 18-21 years, using non-HDL-C

as the preferred lipid measure. We observed that youth non-HDL-C measurements are comparable to LDL-C in predicting preclinical atherosclerosis. However, there is still limited evidence on population-based interventions in youth to reduce cIMT over the long-term. Screening using non-HDL-C comes with the benefit of not requiring measurement of triglycerides to calculate LDL-C and the subsequent advantage of not requiring patients to provide a fasting sample. Concerning the optimal age for lipid screening, our observational data suggest that neither non-HDL-C or LDL-C levels at the age of 9-11 years are associated with subsequent cIMT, as associations only became evident from age 15 years onwards. However, our findings need to be interpreted with caution, as effect estimates were inconsistent across ages. In pooled estimates elevated lipids significantly related with later cIMT only among those aged 18-19 years and dyslipidemic levels only among 15-17-year olds.

For preventive interventions, our findings that the adverse effects of youth dyslipidemia are attenuated if lipid status is improved/normalized by adulthood are encouraging. These data indicate there is a window for change in late adolescence/young adulthood where individual- and public health-focused programs might have long-term preventive effects. In addition, because the effect of elevated childhood non-HDL cholesterol was not completely attenuated/reversed in the resolution group, primordial prevention will continue to be an important goal. We have shown in these cohorts that although lipid levels track, or persist, well from youth to adulthood<sup>34</sup>, those able to change from high risk in youth to normal risk in adulthood coincide with healthful lifestyle changes such as lower gains in fatness and improvements in cardiorespiratory fitness relative to their peers, not smoking, and upward mobility in education<sup>35-38</sup>. Higher gains in BMI from youth to adulthood have been associated with more adverse adult lipid levels irrespective of genetic susceptibility<sup>39</sup>. Indeed, the genetic effect on life-course lipid levels tends to persist, or slightly weaken, with age,

suggesting greater importance of lifestyle factors at different life stages<sup>39</sup>. We have also shown evidence of an infant-onset dietary counselling intervention aimed primarily at improving fat quality in the diet, but also promoting intake of fruit, vegetables, and whole-grains to associate with a higher likelihood of achieving dietary guidelines and with reduced lipid levels even into adulthood<sup>40,41</sup>. These data provide insight on lifestyle and environmental factors that could be targeted at individual or population-wide prevention.

The main strength of this study is the use of pooled data on youth risk factors and adult cIMT from four international longitudinal cohorts. The study also has some limitations. First, because the study cohorts are comprised of relatively young adults at follow-up, we were not able to study associations with cardiovascular events. Instead, we have used cIMT as a surrogate end-point with the risk stratification groupings not based on absolute risk of cardiovascular events (as in adult risk score algorithms), but on high cIMT (defined as  $\geq 90$ th percentile). However, in older adults, cIMT has been shown to predict subsequent CVD events<sup>42</sup>. Second, study participants were predominantly Caucasian, and the results may not be generalizable to other ethnicities. Third, observational studies are prone to bias when trying to establish causality. Finally, concerning the possible confounding factors, most cohorts did not have data available on some possible childhood confounders, such as socioeconomic factors.

In summary, our analysis show that elevated non-HDL-C levels in youth are related to high cIMT in adulthood. In age-stratified analyses, a significant association was observed if non-HDL-C levels were measured at the age of 15-19 years. The predictive utility of youth non-HDL-C and LDL-C were similar. The data also demonstrate that individuals with normal non-HDL-C in youth but elevated non-HDL-C in adulthood had high cIMT, while those with dyslipidemia in youth but normal non-HDL-C as adults had the proportion of high cIMT comparable to those who never had dyslipidemia.

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