Atherosclerosis 341 (2022) 34–42

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Association of adolescent lipoprotein subclass profile with carotid intima-media thickness and comparison to adults: Prospective population-based cohort studies

Raimund Pechlaner [a], Nele Friedrich [b,c], Anna Staudt [d], Nina Gande [d], Benoît Bernar [d,e], Katharina Stock [d,f], Sophia J. Kiechl [a,g], Christoph Hochmayr [d], Andrea Griesmacher [h], Astrid Petersmann [b,i], Kathrin Budde [b], Hermann Stuppner [j], Sonja Sturm [j], Marcus Dörr [c,k], Ulf Schminke [l], Claire Cannet [m], Fang Fang [m], Hartmut Schäfer [m], Manfred Spraul [m], Ralf Geiger [f], Manuel Mayr [n], Matthias Nauck [b,c], Stefan Kiechl [a,g,*], Ursula Kiechl-Kohlendorfer [d], Michael Knoflach [a], for the Early Vascular Ageing (EVA) Study Group1

[a] Department of Neurology, Medical University of Innsbruck, Christoph-Probst-Platz 1, Innrain 52 A, 6020 Innsbruck, Austria
[b] Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany
[c] German Centre for Cardiovascular Research, Partner Site Greifswald, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany
[d] Department of Pediatrics II, Medical University of Innsbruck, Christoph-Probst-Platz 1, Innrain 52 A, 6020 Innsbruck, Austria
[e] Department of Pediatrics I, Medical University of Innsbruck, Christoph-Probst-Platz 1, Innrain 52 A, 6020 Innsbruck, Austria
[f] Department of Pediatrics III, Medical University of Innsbruck, Christoph-Probst-Platz 1, Innrain 52 A, 6020 Innsbruck, Austria
[g] VASCage, Research Centre for Promoting Vascular Health in the Ageing Community, Innrain 66a, 6020 Innsbruck, Austria
[h] Central Institute of Medical and Chemical Laboratory Diagnostics, University Hospital of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria
[i] Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Oldenburg, Rahel-Strauss-Straße 10, 26133 Oldenburg, Germany
[j] Institute of Pharmacy/Pharmacognosy, University of Innsbruck, Innrain 52, 6020 Innsbruck, Austria
[k] Department of Internal Medicine B, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany
[l] Department of Neurology, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany
[m] Bruker BioSpin, Silberstreifen 4, 76287 Rheinstetten, Germany
[n] King's British Heart Foundation Centre, King's College London, 125 Coldharbour Ln, SE5 9NU London, United Kingdom

ARTICLE INFO

Keywords:
Adolescents
Lipoproteins
Intima-media thickness
Prevention
Cholesterol

ABSTRACT

Background and aims: Assessment of comprehensive lipoprotein subclass profiles in adolescents and their relation to vascular disease may enhance our understanding of the development of dyslipidemia in early life and inform early vascular prevention.

Methods: Nuclear magnetic resonance was used to measure lipoprotein profiles, including lipids (cholesterol, free cholesterol, triglycerides, phospholipids) and apolipoproteins (apoB-100, apoA1, apoA2) of 17 lipoprotein subclasses (from least dense to densest: VLDL-1 to −6, IDL, LDL-1 to −6, HDL-1 to −4) in n = 1776 14- to 19-year olds (56.6% female) and n = 3027 25- to 85-year olds (51.5% female), all community-dwelling. Lipoprotein profiles were related to carotid intima-media thickness (cIMT) as ascertained by sonography.

Results: Adolescents compared to adults had lower triglycerides, total, LDL, and non-HDL cholesterol, and apoB, and higher HDL cholesterol. They showed 26.6–59.8% lower triglyceride content of all lipoprotein subclasses and 21.9–51.4% lower VLDL lipid content. Concentrations of dense LDL-4 to LDL-6 were 36.7–40.2% lower, with also markedly lower levels of LDL-1 to LDL-3, but 24.2% higher HDL-1 ApoA1. In adolescents, only LDL-3 to LDL-5 subclasses were associated with cIMT (range of differences in cIMT for a 1-SD higher concentration, 4.8–5.9 μm). The same associations emerged in adults, with on average 97 ± 42% (mean ± SD) larger effect sizes, in addition to LDL-1 and LDL-6 (range, 6.9–11.3 μm) and HDL-2 to HDL-4, ApoA1, and ApoA2 (range, −7.0 to −17.7 μm).

* Corresponding author. Department of Neurology, Medical University of Innsbruck, Anichstraße 35, Innsbruck, Austria.
E-mail address: stefan.kiechl@i-med.ac.at (S. Kiechl).

[1] A complete list of group members appears in the Acknowledgements.

https://doi.org/10.1016/j.atherosclerosis.2021.12.007
Received 26 April 2021; Received in revised form 9 December 2021; Accepted 10 December 2021
Available online 16 December 2021

Conclusions: Adolescents showed a markedly different and more favorable lipoprotein profile compared to adults. Dense LDL subclasses were the only subclasses associated with cIMT in adolescents, implicating them as the potential preferred therapeutic target for primary prevention of cardiovascular disease at this age. In adults, associations with cIMT were approximately twice as large as in adolescents, and HDL-related measures were additionally associated with cIMT.

1. Introduction

Atherosclerosis begins in early life [1]. Coronary arteries of infants may already feature isolated foam cells, and those of children show accumulations of foam cells, lipid droplets, and extracellular lipid [2]. Advanced coronary atheroma was found in 8% of children [2], and severe coronary atherosclerosis in 5% of young adults [3]. At the same time, cumulative exposure to risk factors may still be low in youngers, and potential for prevention correspondingly high. The impact of cumulative risk factor exposure is underscored by Mendelian randomization analyses demonstrating that innate long-term exposure to low low-density lipoprotein (LDL) cholesterol (C) starting in early life confers an approximately 3-fold greater reduction in coronary heart disease risk per unit reduction in LDL-C than does statin therapy initiated later in life [4].

Accordingly, the necessity of early prevention of atherosclerosis manifestation and progression is increasingly being recognized. Current European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines for the management of dyslipidaemias recommend testing of LDL-C in childhood to identify children with familial cholesterolemia, who should receive a modification of lifestyle, including diet, and statin therapy [5]. Current AHA guidelines recommend measuring lipoprotein profiles as early as at 2 years of age if a family history of cardiovascular disease (CVD) or significant hypercholesterolemia are present, and suggest measurement at ages 9 to 11 and again at ages 17 to 21 in lower-risk children and adolescents [6]. High-intensity lifestyle therapy is recommended for youngsters with lipid disorders related to obesity, and statin therapy for some children with very high lipid-related cardiovascular risk such as those with familial hypercholesterolemia [6].

While traditionally, total (T) LDL and high-density lipoprotein (HDL) cholesterol as well as triglycerides (TG) have been used for risk stratification and treatment monitoring, nuclear magnetic resonance allows profiling of the lipid and protein constituents of all lipoprotein subclasses [7], providing a global view of lipid metabolism. Retaining the heterogeneity of subclasses has previously yielded differential associations with cardiovascular end-points within the same lipoprotein class [8], as notably represented by the “atherogenic lipoprotein phenotype” (ALP) featuring a predominance of small dense LDL (sdLDL), lower levels of large HDL, and elevated very-low-density lipoproteins (VLDL) [8].

Associating lipid parameters with incident cardiovascular events in youngsters is difficult due to low event rates. Carotid intima-media thickness (cIMT), while not in itself constituting atherosclerosis [9], is a surrogate marker of vascular disease that independently predicts future myocardial infarction and stroke risk [10] and has previously been successfully used to identify cardiovascular risk factors in youngsters [11,12]. With the caveat that thickness of the tunica media is also reflected in cIMT, introducing a dependence on blood pressure that in adolescents may be more likely due to physiological adaptations rather than arterial hypertension [13], adolescent cIMT has been shown to depend on several established adult cardiovascular risk factors, including total and LDL cholesterol, triglycerides, body-mass index, glycated hemoglobin [14] as well as smoking and lipoprotein(a) [11,12].

Here, for the first time, we report comprehensive, representative adolescent lipoprotein subclass profiles as compared to adult profiles and relate them to cIMT. We find that while adolescents featured more favorable profiles, higher levels of sdLDL were already linked to higher cIMT in adolescents.

2. Patients and methods

2.1. The early vascular aging (EVA) study

The EVA-Tyrol study is a prospective cohort study that aims to assess prevalence of and change in cardiovascular risk profiles in adolescents [15–17]. From the survey area of Tyrol, a state in western Austria with approximately 745 000 inhabitants, and Bruneck, a city in South Tyrol (Italy) with approximately 16 850 inhabitants, female and male students and apprentices in 10th to 12th grade, on average aged 15–17 years, were recruited from local schools, companies and training institutions. We included entire classes rather than recruiting volunteers from several classes of the same school. The baseline assessment, whose data are used in this work, was performed between May 2015 and August 2017 and included, among other measures, anthropometric measurements, assessment of cIMT, blood sampling, and assessment of lifestyle factors, medical history, cardiovascular risk factors, and socioeconomic status by detailed questionnaires. A detailed description of the methods has been published [15]. The EVA-Tyrol study is registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT03929692).

2.2. The study of health in Pomerania (SHIP)

The SHIP is a population-based cohort study conducted in West Pomerania, a region in northeast Germany [18,19]. The two-stage stratified cluster sampling was based on official data from population registries in the Federal State of Mecklenburg/West Pomerania. Study subjects received a maximum of three written invitations, followed by repeated telephone calls and in-person contacts at home. Baseline examinations were performed between 1997 and 2001 in 4308 men and women aged 20–79 years (response rate 68.8%). The first follow-up (SHIP-1) was performed between 2002 and 2006 with 3300 subjects being re-examined (response rate 83.6%). Nuclear magnetic resonance (NMR) measurements and cIMT measurement were performed in 3156 participants, and after exclusion of subjects with missing values in adjustment variables 3027 remained.

2.3. Assessment of cardiovascular risk factors and anthropometric parameters

In the EVA study, Family Affluence score was employed as a measure of social status [20], alternative healthy eating index [21] as a measure of diet quality, and leisure-time physical activity was assessed using the Baecke questionnaire [22]. Cigarette smoking was defined as regular smoking of at least one cigarette per week and amount smoked was ascertained in a physician interview. Alcohol consumption was similarly determined by interview, and grams of alcohol consumed was derived by multiplying alcohol content of beverages with intake frequency.

Blood samples were taken after an overnight fast and immediately cooled, and laboratory parameters measured within 24 h after venipuncture. Total cholesterol, LDL-C, HDL-C, and TG were measured by direct enzymatic colorimetric assays (Cobas 8000 system, Roche/Hitachi, Vienna, Austria). Serum glucose was assessed by the hexokinase method (Roche, Cobas 8000).

Weight was determined using calibrated medical precision scales,

height by a Harpenden stadiometer (Holtain, Crymych, United Kingdom). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m2). Blood pressure was measured in the seated position after a 5-min resting period, with measurements taken 3 times on the left or right upper arm with the appropriate cuff size using an automated oscillometric device (Omron M4-I; Omron Healthcare, Lake Forest, Illinois).

In the SHIP, all participants underwent standardized medical examinations, blood sampling, and an extensive computer-aided personal interview. Data on socio-demographic characteristics (e.g. c school education or income) and medical histories were collected. Smoking status was assessed by self-report and categorized into current, former, and never-smokers. Regular physical activity per week was categorized into three groups (no activity, <2 h/week or ≥ 2 h/week). Alcohol consumption was assessed using the drink-specific quantity–frequency (Q–F) measure: number of days with alcohol consumption and average daily alcohol consumption for such a day over the past month. Average alcohol consumption (in grams per day) was calculated by multiplying frequency and amount of alcohol from beer, wine, and spirits, respectively, using a standard ethanol content of 4.8% (by volume) in beer, 11% (by volume) in wine and 33% (by volume) in spirits to conversion. Intake of medication was recorded and classified using the anatomical therapeutic chemical classification system (ATC).

During the physical examination, standardized measurements of body height and weight were performed with calibrated scales. BMI was calculated as weight (kg)/height^2 (m^2). After a resting period of at least 5 min, systolic and diastolic blood pressure were measured three times on the right arm of seated participants with an oscillometric digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan). The interval between the readings was 3 min. The mean of the second and third measurements was calculated. Non-fasting blood samples were drawn from the cubital vein in the supine position. The samples were analyzed immediately or stored at −80 °C.

2.4. Lipoprotein subfraction measurements

Lipoprotein subfraction parameters were measured using nuclear magnetic resonance at Bruker BioSpin GmbH Rheinstetten (EVA cohort) and at Greifswald University (SHIP cohort). The measurement protocol is described in detail in the Supplemental Material, and analytes measured are listed in Supplemental Table 3. In brief, blood samples were immediately stored after sampling in cooling bags at 4–8 °C and centrifuged within 3 h, then stored at −70 °C until analysis, which utilized 300 μl of serum taken in the fasting state in EVA, and 250 μl of EDTA-plasma taken in the non-fasting state in SHIP. Neither pre-analytic nor analytic variables were included in statistical models.

For the utilized NMR methods, lipoprotein measurements in plasma and in serum have been shown to exhibit excellent agreement, with an overall correlation and root mean squared error of 0.999 and 1.5 mg/dL, respectively [7]. For individual LDL 1–6 subclasses, correlations were in the range of 0.966–0.985, and root mean squared error within 1.9–2.6 mg/dL.

An assay version earlier than in EVA was employed in SHIP, thus total FC and VLDL-6 TG, C, FC, and PL were available only in the former.

2.5. Assessment of cIMT

In the EVA study, cIMT was assessed by high-resolution ultrasound with the use of a 6.0–13.0 MHz 14 × 47 mm linear probe on a GE healthcare Vivid q (General Electric Healthcare, Wauwatosa, Wisconsin, USA). Participants were examined in the supine position. First, the common carotid artery was displayed and the beginning of the carotid bulb was defined as the location where the near and far wall begin to diverge. Visualization of the cIMT was done in the lateral position. Representative images were stored in the DICOM format. Greatest far wall thickness of the most distal 4 cm of the common carotid artery of both sides in longitudinal images was identified. Three representative measurements were taken per side at different locations where the border reflexes were clearly visible over at least 10 mm. The maximum of all six measurements was used as the final measure of cIMT. All measurements were carried out on digitally stored images by a single rater blinded to the clinical characteristics of the study participants.

In the SHIP-1, the examination of the extracranial carotid arteries was performed with B-mode ultrasonography using a 5 MHz linear array transducer with an axial resolution of less than 0.5 mm and a high-resolution instrument (Diasonics VST Gateway, Santa Clara, USA). The maximal far-wall IMT was calculated from ten measurements taken manually in 1-mm steps from off-line scans of the distal straight portion of both common carotid arteries.

2.6. Statistical analysis

Baseline characteristics are presented as count (percentage), mean ± standard deviation, or median (interquartile range). Proposed reference ranges of lipoprotein subclass parameters were calculated as the respective 5th and 95th percentile in both adolescents and adults. Agreement of NMR-based measurements with clinical routine measurements was investigated using Pearson correlation and agreement-based intraclass correlation [23]. Differences in lipoprotein profiles between adolescents and adults were investigated using the Welch *t*-test, and effects are shown as crude percent difference. Associations of lipoprotein parameters with cIMT were examined using linear regression, adjusting for age, sex, body-mass index (in continuous form), systolic and diastolic blood pressure, smoking, oral contraception, and physical activity in both EVA and SHIP, and additionally for diet quality, type of schooling and social status [20] in the EVA study, and for income and education in the SHIP. Effect sizes for associations with cIMT are given for 1-SD higher levels based on SDs in the EVA study to facilitate a direct comparison of EVA *vs.* SHIP, and effect sizes on the native SHIP SD scale are shown separately for SHIP. Differential effects by sex were tested by including an appropriate interaction term between each lipid variable and sex. Adjustment of significance levels for multiple testing was performed using the Benjamini-Hochberg procedure [24], and results are deemed statistically significant based on a q-value cut-off of 0.05. Analysis was conducted using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

2.7. Ethics approval

Both the EVA and the SHIP study were conducted in compliance with the Declaration of Helsinki and approved by the Institutional Review Board of the Medical University of Innsbruck (approval AN 2015-0005 345/4.13) or the Ethics Committee of the University of Greifswald (Reg.-Nr. III UV 73/01). Written informed consent was obtained from all participants in both studies, and from the legal guardians of participants of the EVA study where applicable.

3. Results

3.1. Characteristics of the study cohorts and lipoprotein measurements

Lipoprotein measurements were available for n = 1776 14- to 19-year olds (mean age, 16.4 years; 43.4% male) and for n = 3027 25- to 85-year olds (mean age, 55.0 years; 48.5% male) (Table 1). Detailed information on absolute levels and reference ranges of lipoprotein measurements are provided in Supplemental Table 1.

Comparing measurements in adolescents based on NMR-based assays with those of routine clinical assays yielded Pearson correlations (95% confidence intervals) of 0.96 (0.95, 0.96), 0.91 (0.90, 0.92), 0.94 (0.93, 0.94), and 0.97 (0.96, 0.97) and intraclass correlation coefficients of 0.88 (0.87, 0.89), 0.89 (0.89, 0.90), 0.91 (0.90, 0.92), and 0.94 (0.94, 0.95) for total cholesterol, LDL-C, HDL-C, and TG, respectively,

Table 1
Characteristics of the study cohorts.

Variable	EVA	SHIP
n	1776	3027
Male sex	771 (43.4)	1469 (48.5)
Age, years	16.4 ± 1.1	54.2 ± 15.1
Total cholesterol, mg/dL	159 ± 29	203 ± 41
HDL-C, mg/dL	58.6 ± 13.4	48.7 ± 14.9
LDL-C, mg/dL	93.1 ± 25.3	122.4 ± 33.5
NonHDL-C, mg/dL	100 ± 27	154 ± 42
TG, mg/dL	70.0 (39.0)	164.9 (97.5)
TG category		
<150 mg/dL	1670 (94.1)	1539 (54.1)
≥150 mg/dL	105 (5.9)	1303 (45.9)
Lipid modifying therapy		
None	1776 (100.0)	2586 (85.4)
Statins	0 (0.0)	397 (13.1)
Fibrates	0 (0.0)	28 (1.0)
Bile acid sequestrants	0 (0.0)	3 (0.1)
Other	0 (0.0)	17 (0.6)
Statins in combination with other	0 (0.0)	3 (0.1)
Alcohol intake, grams/day	1.4 (7.1)	9.9 (14.6)
Diastolic BP, mmHg	71.2 ± 7.7	81.4 ± 10.6
Systolic BP, mmHg	123 ± 12	132 ± 20
Fasting glucose, mg/dL	77.1 ± 9.1	
Smoking		
Current	431 (24.3)	771 (25.5)
Former	98 (5.5)	993 (32.8)
Never	1247 (70.2)	1263 (41.7)
Body-mass index, kg/m^2	21.3 (4.1)	27.9 (6.8)
Body-mass index category		
<18.5	241 (13.6)	16 (0.5)
18.5–25	1262 (71.3)	877 (29.0)
25–30	212 (12.0)	1230 (40.6)
30–40	53 (3.0)	853 (28.2)
≥40	2 (0.1)	51 (1.7)
Oral contraceptive use[a]		
Absent	751 (75.4)	1413 (90.7)
Present	245 (24.6)	145 (9.3)
School type		
EVA: Apprenticeship; SHIP: <10 years	235 (13.2)	1173 (38.8)
EVA: High school; SHIP: 10 years	595 (33.5)	1319 (43.6)
EVA: Vocational school; SHIP: >10 years	946 (53.3)	535 (17.7)
Carotid intima-media thickness, μm	488 ± 60	889 ± 258

Characteristics are presented as count (percentage), mean ± standard deviation, or median (interquartile range).
HDL, high-density lipoprotein; C, cholesterol; LDL, low-density lipoprotein; TG, triglycerides; BP, blood pressure; Fasting glucose was not available in SHIP.
[a] Reported only for women.

indicating high agreement.

3.2. Comparison of adolescent to adult lipoprotein profile

Adolescents had lower levels of T-TG, T-C, and T-apoB, lower non-HDL-C, and higher HDL-C (Fig. 1). They showed 26.6–59.8% lower TG content of all lipoprotein subclasses, with the difference most pronounced for IDL, less dense VLDL, and denser LDL. Concentrations of all lipids within VLDL subclasses as well as overall VLDL apoB content were 21.9–51.4% lower in adolescents. Reductions in dense LDL-4, LDL-5, and LDL-6 were most pronounced (36.7–40.2%), with lesser reductions also of LDL-1 to LDL-3 (5.6–21.7%). The least dense HDL-1 featured 23.3 and 24.1% higher contents of FC and ApoA-I, while, apart from reductions in TG of 26.6–36.8%, other effects in HDL subclasses were weaker.

3.3. Association of the adolescent lipoprotein profile with cIMT and comparison to adults

Significant associations with cIMT in adolescents emerged for T-C and apoB (difference in cIMT for a 1-SD higher concentration: 3.9 and 4.8 μm) (Fig. 2 and Supplemental Table 2). The largest associations were found for dense LDL-3, LDL-4, and LDL-5 subclasses (difference for a 1-SD higher concentration, 4.8–5.9 μm), whereas no significant association with cIMT was observed for any VLDL-, IDL- or HDL-related variable. There was no evidence of differential association with cIMT by sex for any lipoprotein measurement (all $q > 0.05$).

All the parameters associated with cIMT in EVA were also associated with cIMT in SHIP, with 41–193% (mean ± SD: 97 ± 42%) larger effect sizes (Fig. 2 and Supplemental Table 2). Associations present in SHIP but not EVA included direct associations with cIMT of ApoB on IDL, LDL-1, and LDL-6, with differences in cIMT per 1-SD higher concentration of IDL or LDL subclasses of 5.7–11.7 μm. Associations of TG on total LDL as well as LDL-2, -3, and -5 subclasses were also restricted to SHIP, and differences in cIMT associated with LDL-TG were in the range of 6.9–11.3 μm. Inverse associations with cIMT exclusive to SHIP included those of total HDL and HDL-2, -3, and -4 C, FC, and PL, and T- and HDL-ApoA1 and ApoA2 as well as ApoA1 in all 4 HDL subclasses, with cIMT differences of −7.0 to −17.7 μm. No significant associations emerged for any VLDL-related measures in EVA or SHIP.

Standard deviations of all lipoprotein parameters were on average 64 ± 37% (range: 25–138%) larger in SHIP than in EVA (Supplemental Table 1), and for lipoprotein parameters significantly associated with cIMT in SHIP, SDs were 48 ± 22% larger.

4. Discussion

Comprehensive NMR-based profiling of lipoprotein subfractions in

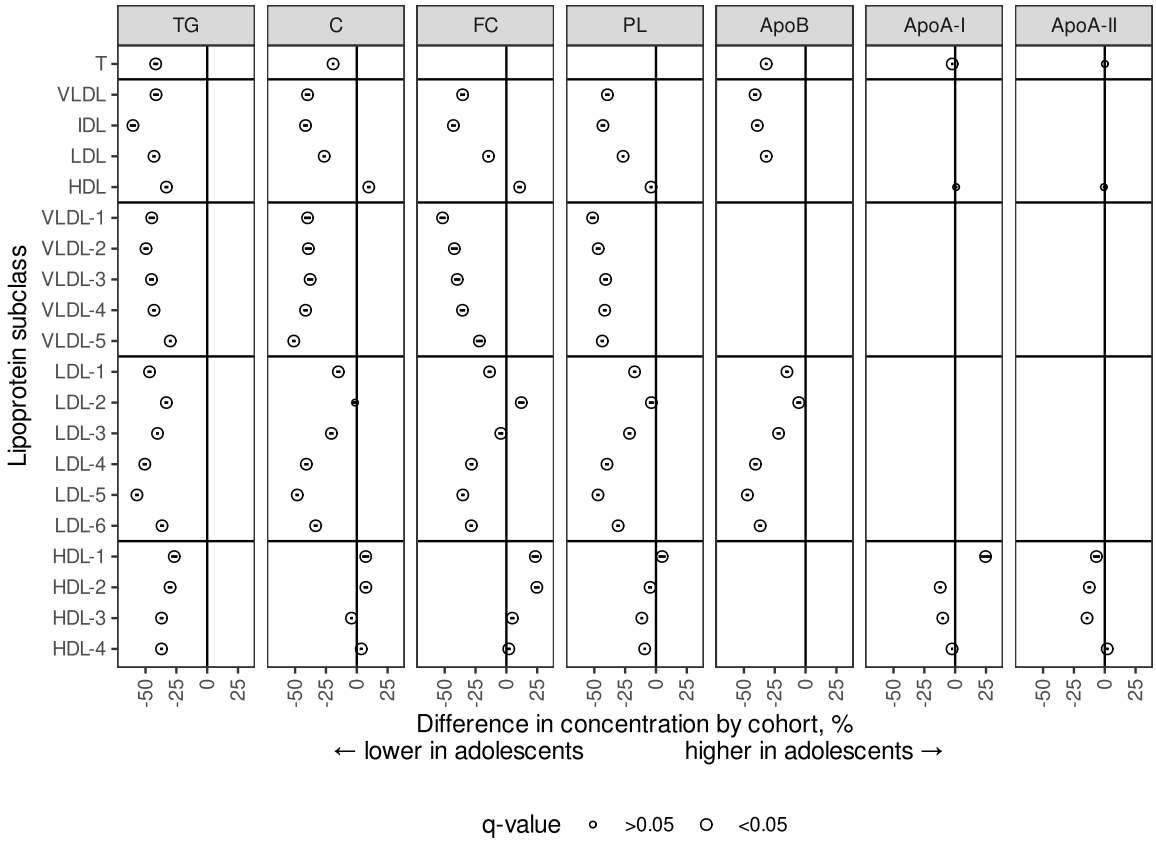

Fig. 1. Differences in lipoprotein subclass profiles between adolescents and adults.
Percent differences between adolescent subjects in the EVA study and adult subjects in the SHIP study of 101 lipoprotein parameters are shown as points, and confidence intervals as horizontal segments (due to small width contained within points). Significant results ($q < 0.05$) are marked by large points. Compartment (total, classes, and subclasses) is shown on the y-axis, and lipid and protein constituents are shown in panels. Within subclasses, density increases with increasing number, with 1 representing the least dense particles. Adolescents featured lower total TG, C, and apoB, lower non-HDL-C, and higher HDL-C. Triglyceride content of all lipoprotein subclasses was lower, as well as lipid content of all VLDL subclasses. Reductions in denser LDL-4, LDL-5, and LDL-6 were most pronounced, with lesser reductions also of LDL-3 and LDL-1. The least dense HDL-1 featured higher contents of C, FC, and ApoA-I, while other effects in HDL subclasses were weaker. T, total; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; C, cholesterol; FC, free cholesterol; PL, phospholipids; ApoB, apolipoprotein B-100; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; Further detail is given in Supplemental Table 1.

two large cohorts yields the following major conclusions: (1) Adolescents showed lower concentrations than adults of most lipid measures in most subclasses, with differences most pronounced for TG contents, VLDL subclasses, denser LDL subclasses, and apoB. (2) Denser LDL subclasses and apoB, but not VLDL-, IDL- or HDL-related measures were associated with cIMT in adolescents. (3) Associations of lipoprotein parameters with cIMT in adults included all those found in adolescents and extended to HDL, and effect sizes were larger than in adolescents in all cases.

The different lipid and protein constituents of individual apoB-containing subclasses largely showed concordant effects in analyses, suggesting that variation in particle size or number had a stronger impact than variation in particle composition. As the employed lipoprotein subfractionation by density is expected to result in decreased size variation within each lipoprotein subclass, variation in particle number may have been the more important factor. ApoB is proportional to particle number because each VLDL, IDL and LDL particle contains exactly one apoB molecule.

4.1. Adolescent lipoprotein profile, association with cIMT, and comparison to adults

Adolescents from the EVA study compared to adults from the SHIP study showed a more favorable lipoprotein profile, including lower T-TG, T-C, and T-apoB, lower non-HDL-C, reductions in denser LDL subclasses, and higher HDL-C (Fig. 1 and Supplemental Table 1).

Larger as well as smaller LDL subfractions have previously been shown to be significantly associated with incident CVD [25] and carotid vasculopathy [26], while small dense LDL have been implicated in the etiology of coronary heart disease [27] due to specific characteristics including longer residence in the circulation and higher permeability through the endothelial barrier [28]. Small dense LDL is one component of a host of interrelated metabolic risk factors also including hypertriglyceridemia and low HDL-C, as reflected in the traditional atherogenic lipoprotein phenotype concept [8,29]. Adolescents in this study showed more favorable levels of all of these risk factors than adults. Associations with cIMT in adolescents emerged for T and LDL apoB and C, as well as for denser LDL subclasses (LDL-3, -4 and -5) (Fig. 2). In adults, all of these lipoprotein parameters were likewise associated with cIMT, in addition to LDL-1 and LDL-6-related parameters.

Lower TG content of all measured lipoprotein subfractions in adolescents (Fig. 1) may be due to known decrease of lipoprotein lipase, the enzyme responsible for delipidation of triglyceride-rich lipoproteins, with age [30]. Although sampling of blood in the fasted state, as was performed in adolescents, results in approximately 26 mg/dL lower total triglycerides as compared to non-fasted sampling [31], as was performed

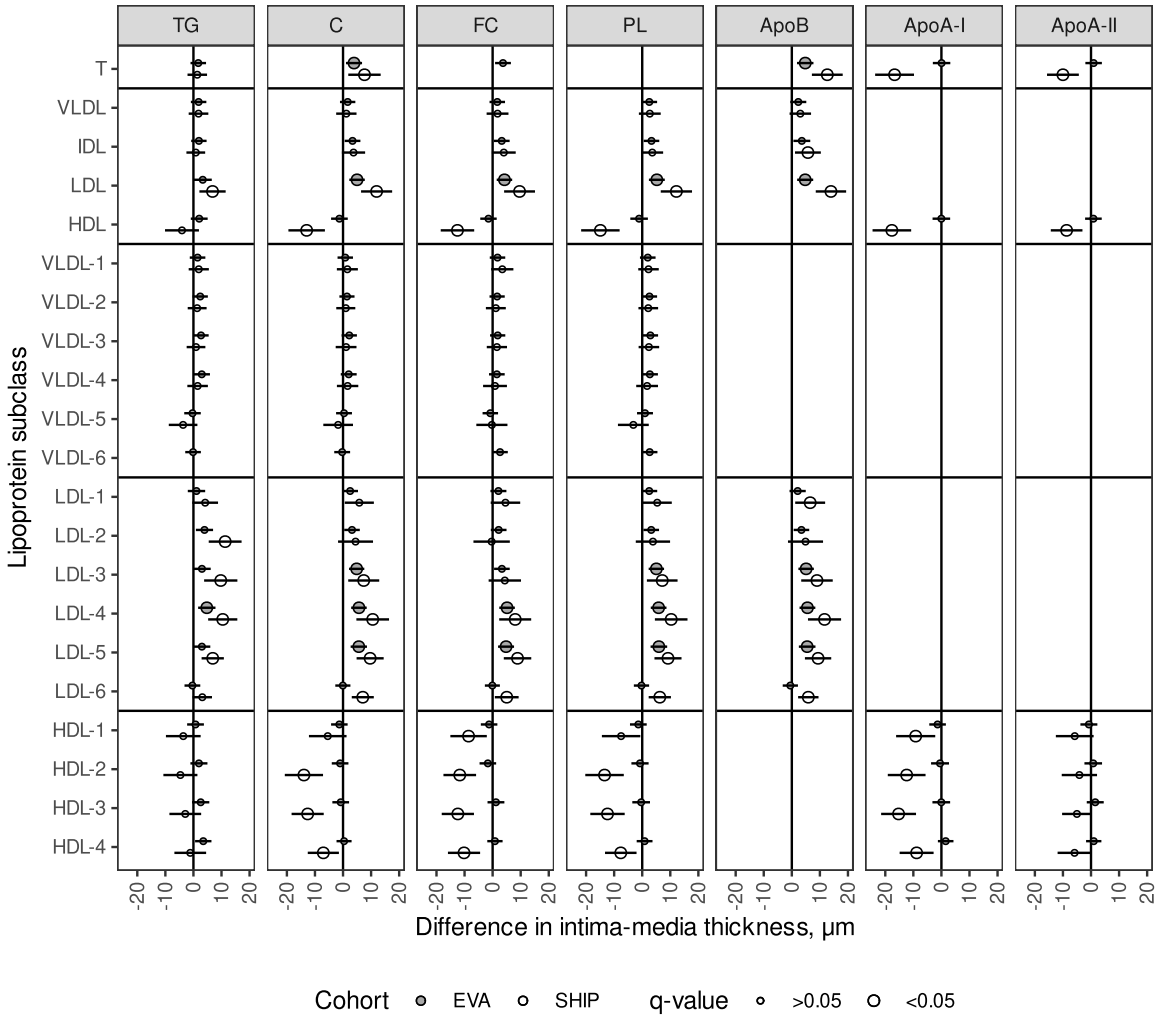

Fig. 2. Associations of lipoprotein subclass profiles with carotid intima-media thickness in adolescents (EVA study) and adults (SHIP study).
Differences in carotid intima-media thickness (µm) associated with 1-SD higher levels of lipoprotein subfraction parameters are shown as points, and corresponding 95% confidence intervals as horizontal segments. Significant results ($q < 0.05$) are marked by large points. Compartment (total, classes, and subclasses) is shown on the y-axis, and lipid and protein constituents are shown in panels. Within subclasses, density increases with increasing number, with 1 representing the least dense particles. In the EVA study, T-C and -apoB and most LDL-related parameters were significantly associated with cIMT, while within subclasses only the denser LDL-3, -4, and -5 showed significant associations. All of these parameters were also significantly associated with cIMT in SHIP, and with larger effect sizes in all cases. Associations with cIMT present in SHIP but not EVA included direct associations of ApoB on IDL, LDL-1, and LDL-6 and of TG on LDL as well as LDL-2, -3, and -5 subclasses, and inverse associations of total HDL and HDL-2, -3, and -4 C, FC, and PL, and T- and HDL-ApoA1 and ApoA2 as well as ApoA1 in all 4 HDL subclasses. Results are adjusted for age, sex, body-mass index, systolic and diastolic blood pressure, smoking, oral contraception, and physical activity in both EVA and SHIP, and additionally for diet quality, type of schooling and social status in the EVA study, and for income and education in the SHIP study. Effects are scaled to correspond to 1-SD increases based on EVA SDs. Detailed results are provided in Supplemental Table 2. T, total; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; C, cholesterol; FC, free cholesterol; PL, phospholipids; ApoB, apolipoprotein B-100; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; SD, standard deviation.

in adults, the difference we observed (78 *vs.* 129 mg/dL total triglycerides, Supplemental Table 1) was approximately twice as large and cannot be explained by fasting state alone. Lower levels of VLDL, the primary TG-laden lipoprotein, in adolescents are considered favorable based on reported atherogenic effects of their remnants [32,33]. Importantly, adolescent VLDL-C were lower than those of adults across the VLDL density spectrum, which may be viewed as beneficial because cholesterol has been reported as the atherogenic agent [32] in VLDL and their remnants. However, in the current study, only LDL-TG but not TG in other compartments or any VLDL-related measure showed significant associations with cIMT (Fig. 2 and Supplemental Table 2), which were restricted to TG in LDL-4 in EVA and included TG in LDL-2 to −5 in SHIP. These associations might reflect associations of LDL more than effects specific to LDL-TG because TG is only a minor component of LDL, and the pattern of association was similar as for other LDL constituents with a preponderance of intermediate to high density subclasses (Fig. 2). A potential explanation for lack of associations with cIMT of TG or VLDL beyond LDL-TG in adolescents lies in their low TG levels, as a quantitative threshold for injurious effects may be present, in contrast to LDL for which the association with vascular risk follows a principle of "the lower the better" down to very low LDL levels [34]. In adults, this lack of association is somewhat unexpected because triglyceride-rich lipoproteins have been causally implicated in cardiovascular disease [32,35,36]. As VLDL may be more dependent on lifestyle, particularly diet [37], than other lipoprotein parameters, statistical power may have been reduced by the comprehensive multivariable adjustment we employed. The association of VLDL-C with cIMT was found non-significant under comprehensive adjustment in prior studies in adults [38] and adolescents [39].

Differences in HDL between adolescents and adults were less uniform for the various subclasses. For the least dense HDL-1, higher content of FC and ApoA-I may indicate higher levels of these lipoproteins in adolescents. Efflux capacity, the capacity of HDL to accept cholesterol from macrophages, is inversely associated with incident cardiovascular events [40] and is directly associated with large, less dense HDL and inversely associated with small, denser HDL [41]. Only weak differences between adolescents and adults emerged for other HDL subclasses, with the exception of markedly lower triglyceride content in all HDL subclasses in adolescents. Triglyceride-rich HDL are one component of the dyslipidaemia of insulin resistance and type 2 diabetes [42]. Lack of any significant associations between HDL and cIMT in adolescents may partly reflect a similar phenomenon as was observed for TG, as HDL-C is inversely correlated with TG [43] and HDL-related lipoprotein parameters were lower than in adults (Supplemental Table 1). In adults from the SHIP study, large associations emerged between cIMT and HDL subclasses (Fig. 2 and Supplemental Table 1), in line with established inverse associations between HDL and cardiovascular risk.

While effect sizes in adolescents of approximately 4–6 μm higher cIMT per 1-SD higher levels of LDL subclass parameters are of roughly similar magnitude as previously found in the Atherosclerosis Risk in Young Adults study in on average 13-year old adolescents for body-mass index (~8 μm/SD) and systolic blood pressure (~5 μm/SD) [44], effect sizes for LDL or LDL subclasses in adults from SHIP on the SHIP SD scale of 9–20 μm/SD (Supplemental Table 2) are smaller than those reported previously in the Multi-Ethnic Study of Atherosclerosis (average age, 61 years) of 32 μm/SD [26]. Part of this discrepancy may derive from more comprehensive multivariable adjustment employed here, attenuating estimated effects. Importantly, for comparison to adolescents in EVA we scaled results in adults from SHIP to the EVA SD scale, as SDs were approximately 50% higher in adults than in adolescents. Based on this direct comparison we found associations of lipoprotein parameters with cIMT in adults with effect sizes on average twice as large as in adolescents, possibly reflecting different durations of risk factor exposure and underscoring the substantial potential for prevention in youngsters [26].

4.2. *Strengths, limitations, and future directions*

Strengths of this study include its large sample size and the use of standardized and validated lipoprotein assays [7] performed on Bruker IVDr Systems for both adolescents and adults. Adolescent participants were sampled from all regions of the Tyrol and from all different types of school or apprenticeship and may thus be viewed as representative of Tyrolean adolescents. To the best of our knowledge, this is the first study that reports comprehensive adolescent nuclear magnetic resonance lipoprotein subclass profiles as compared to adults, and their association with cIMT.

One limitation is that we did not include pubertal stage, even though almost all participants are expected to have been in Tanner stage IV or V [45], and previous evaluations of vasculopathy in 13-year old adolescents did not find an influence of Tanner stage [44,46]. A further limitation pertains to the use of fasting samples in the EVA, but non-fasting samples in the SHIP study, which may have resulted in lower triglyceride measurements as discussed above, approximately 8 mg/dL higher T-C, LDL-C, and non-HDL-C, and no significant difference in apoB and HDL-C, in the EVA study [31]. While fasting lipid profiles represent the traditional standard for cardiovascular disease prevention, non-fasting measurements are increasingly recognized as equivalent [31,47].

Future studies could extend on the current by investigating adolescent lipid composition at the level of lipid species and adolescent apolipoprotein profiles, providing a complementary view of the relation between the lipidome and cardiovascular risk [35,48], and by testing the effect of modifying lipoprotein profiles in adolescents on the occurrence of cardiovascular disease.

In summary, adolescents showed markedly different and favorable lipoprotein profiles as compared to adults, but denser LDL subclasses were already associated with subclinical vascular disease. These findings inform on adolescent lipoprotein metabolism and may aid primary prevention of cardiovascular disease at this age. From a public health perspective, our findings might suggest lowering of sdLDL to reduce cardiovascular risk in adolescents, possibly by reducing dietary intake of simple carbohydrates and increasing physical exercise [49].

Clinical trial registration

The EVA-Tyrol study is registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT03929692).

Financial support

This work was supported by the excellence initiative VASCage (Centre for Promoting Vascular Health in the Ageing Community) [project number 868624] of the Austrian Research Promotion Agency FFG (COMET program–Competence Centers for Excellent Technologies) funded by the Austrian Ministry for Transport, Innovation and Technology; the Austrian Ministry for Digital and Economic Affairs; and the federal states Tyrol (via Standortagentur), Salzburg, and Vienna (via Vienna Business Agency). Prof Manuel Mayr is a British Heart Foundation (BHF) Chair Holder [CH/16/3/32 406] with BHF programme grant support [RG/16/14/32 397]. This work was supported by the National Institute of Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' National Health Service (NHS) Foundation Trust and King's College London in partnership with King's College Hospital. SHIP is supported by the German Federal Ministry of Education and Research [Grants 01ZZ0403, 01ZZ0103, 01ZZ9603], the German Ministry for Education, Research and Cultural Affairs, as well as the Ministry of Social Affairs of the Federal State of Mecklenburg-West Pomerania.

CRediT authorship contribution statement

Raimund Pechlaner: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Nele Friedrich:** Data curation, Formal analysis, Resources, Software, Writing – review & editing. **Anna Staudt:** Data curation, Investigation, Resources, Writing – review & editing. **Nina Gande:** Data curation, Investigation, Resources, Writing – review & editing. **Benoît Bernar:** Data curation, Investigation, Resources, Writing – review & editing. **Katharina Stock:** Data curation, Investigation, Resources, Writing – review & editing. **Sophia J. Kiechl:** Data curation, Investigation, Resources, Writing – review & editing. **Christoph Hochmayr:** Data curation, Investigation, Resources, Writing – review & editing. **Andrea Griesmacher:** Data curation, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Astrid Petersmann:** Data curation, Investigation, Resources, Writing – review & editing. **Kathrin Budde:** Data curation, Investigation, Resources, Writing – review & editing. **Hermann Stuppner:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Marcus Dörr:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Ulf Schminke:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Claire Cannet:** Data curation, Investigation, Resources, Writing – review & editing. **Fang Fang:** Data curation, Investigation, Resources, Writing – review & editing. **Hartmut Schäfer:** Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing, Investigation, Resources, Writing – review & editing. **Manfred Spraul:** Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. **Ralf Geiger:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Manuel Mayr:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Matthias Nauck:**

Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Stefan Kiechl:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ursula Kiechl-Kohlendorfer:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Michael Knoflach:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

EVA study group members: Anna Staudt, Benoît Bernar, Bernhard Winder, Carmen Reiter, Christina Burger, Christoph Hochmayr, Gregor Brössner, Hermann Stuppner, Julia Klingenschmid, Julia Marxer, Katharina Stock, Mandy Asare, Manuel Mayr, Manuela Bock-Bartl, Martina Kothmayer, Maximilian Bohl, Maximilian Pircher, Michael Knoflach, Nina Gande, Raimund Pechlaner, Ralf Geiger, Sonja Sturm, Sophia J. Kiechl, Stefan Kiechl, Tatjana Heisinger, Ursula Kiechl-Kohlendorfer.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2021.12.007.

References

[1] Y.M. Hong, Atherosclerotic cardiovascular disease beginning in childhood, Korean Circ. J. 40 (2010) 1–9, https://doi.org/10.4070/kcj.2010.40.1.1.
[2] H.C. Stary, Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults, Arterioscler. Dallas Tex. 9 (1989) I19–I32.
[3] J.J. McNamara, M.A. Molot, J.F. Stremple, R.T. Cutting, Coronary artery disease in combat casualties in Vietnam, JAMA 216 (1971) 1185–1187, https://doi.org/10.1001/jama.1971.03180330061012.
[4] B.A. Ference, W. Yoo, I. Alesh, N. Mahajan, K.K. Mirowska, et al., Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a mendelian randomization analysis, J. Am. Coll. Cardiol. 60 (2012) 2631–2639, https://doi.org/10.1016/j.jacc.2012.09.017.
[5] F. Mach, C. Baigent, A.L. Catapano, K.C. Koskinas, M. Casula, et al., 2019 ESC/EAS Guidelines for the Management of Dyslipidaemias: Lipid Modification to Reduce Cardiovascular risk The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS), Eur. Heart J. (n.d.). https://doi.org/10.1093/eurheartj/ehz455.
[6] S.M. Grundy, N.J. Stone, A.L. Bailey, C. Beam, K.K. Birtcher, et al., AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American heart association task force on clinical practice guidelines, Circulation 139 (2018) e1082–e1143, https://doi.org/10.1161/CIR.0000000000000625, 2019.
[7] B. Jiménez, E. Holmes, C. Heude, R.F. Tolson, N. Harvey, et al., Quantitative lipoprotein subclass and low molecular weight metabolite analysis in human serum and plasma by 1H NMR spectroscopy in a multilaboratory trial, Anal. Chem. 90 (2018) 11962–11971, https://doi.org/10.1021/acs.analchem.8b02412.
[8] R.M. Krauss, Lipoprotein subfractions and cardiovascular disease risk, Curr. Opin. Lipidol. 21 (2010) 305, https://doi.org/10.1097/MOL.0b013e32833b7756.
[9] Spence J. David, Carotid ultrasound phenotypes are biologically distinct, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 1910–1913, https://doi.org/10.1161/ATVBAHA.115.306209.
[10] R.T. Hurst, D.W.C. Ng, C. Kendall, B. Khandheria, Clinical use of carotid intima-media thickness: review of the literature, J. Am. Soc. Echocardiogr. Off. Publ. Am. Soc. Echocardiogr. 20 (2007) 907–914, https://doi.org/10.1016/j.echo.2007.02.028.
[11] M. Knoflach, S. Kiechl, M. Kind, M. Said, R. Sief, et al., Cardiovascular risk factors and atherosclerosis in young males: ARMY study (Atherosclerosis Risk-Factors in Male Youngsters), Circulation 108 (2003) 1064–1069, https://doi.org/10.1161/01.CIR.0000085996.95532.FF.
[12] M. Knoflach, S. Kiechl, D. Penz, A. Zangerle, C. Schmidauer, et al., Cardiovascular risk factors and atherosclerosis in young women: atherosclerosis risk factors in female youngsters (ARFY study), Stroke 40 (2009) 1063–1069, https://doi.org/10.1161/STROKEAHA.108.525675.
[13] S.T. Chiesa, M. Charakida, G. Georgiopoulos, F. Dangardt, K.H. Wade, et al., Determinants of intima-media thickness in the young: the ALSPAC study, JACC Cardiovasc. Imaging (2019), https://doi.org/10.1016/j.jcmg.2019.08.026.
[14] J.D. Dawson, M. Sonka, M.B. Blecha, W. Lin, P.H. Davis, Risk factors associated with aortic and carotid intima-media thickness in adolescents and young adults: the Muscatine Offspring Study, J. Am. Coll. Cardiol. 53 (2009) 2273–2279, https://doi.org/10.1016/j.jacc.2009.03.026.
[15] B. Bernar, N. Gande, K.A. Stock, A. Staudt, R. Pechlaner, et al., The Tyrolean early vascular ageing-study (EVA-Tyrol): study protocol for a non-randomized controlled trial : effect of a cardiovascular health promotion program in youth, a prospective cohort study, BMC Cardiovasc. Disord. 20 (2020) 59, https://doi.org/10.1186/s12872-020-01357-9.
[16] K. Stock, A. Schmid, E. Griesmaier, N. Gande, C. Hochmayr, et al., The impact of being born preterm or small for gestational age on early vascular aging in adolescents, J. Pediatr. 201 (2018) 49–54. https://doi.org/10.1016/j.jpeds.2018.05.056.
[17] K. Stock, R. Nagrani, N. Gande, B. Bernar, A. Staudt, et al., Birth weight and weight changes from infancy to early childhood as predictors of body mass index in adolescence, J. Pediatr. (2020), https://doi.org/10.1016/j.jpeds.2020.03.048.
[18] U. John, B. Greiner, E. Hensel, J. Lüdemann, M. Piek, et al., Study of Health in Pomerania (SHIP): a health examination survey in an east German region: objectives and design, Sozial- Präventivmed. 46 (2001) 186–194, https://doi.org/10.1007/BF01324255.
[19] H. Völzke, D. Alte, C.O. Schmidt, D. Radke, R. Lorbeer, et al., Cohort profile: the study of health in Pomerania, Int. J. Epidemiol. 40 (2011) 294–307, https://doi.org/10.1093/ije/dyp394.
[20] W. Boyce, T. Torsheim, C. Currie, A. Zambon, The family affluence scale as a measure of national wealth: validation of an adolescent self-report measure, Soc. Indicat. Res. 78 (2006) 473–487, https://doi.org/10.1007/s11205-005-1607-6.
[21] M.L. McCullough, D. Feskanich, M.J. Stampfer, E.L. Giovannucci, E.B. Rimm, et al., Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance, Am. J. Clin. Nutr. 76 (2002) 1261–1271.
[22] J.A. Baecke, J. Burema, J.E. Frijters, A short questionnaire for the measurement of habitual physical activity in epidemiological studies, Am. J. Clin. Nutr. 36 (1982) 936–942.
[23] J.J. Bartko, The intraclass correlation coefficient as a measure of reliability, Psychol. Rep. 19 (1966) 3–11, https://doi.org/10.2466/pr0.1966.19.1.3.
[24] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B Methodol. 57 (1995) 289–300.
[25] D. Shiffman, J.Z. Louie, M.P. Caulfield, P.M. Nilsson, J.J. Devlin, et al., LDL subfractions are associated with incident cardiovascular disease in the Malmö Prevention Project Study, Atherosclerosis 263 (2017) 287–292, https://doi.org/10.1016/j.atherosclerosis.2017.07.003.
[26] S. Mora, M. Szklo, J.D. Otvos, P. Greenland, B.M. Psaty, et al., LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA), Atherosclerosis 192 (2007) 211–217. https://doi.org/10.1016/j.atherosclerosis.2006.05.007.
[27] F.M. Sacks, H. Campos, Low-density lipoprotein size and cardiovascular disease: a reappraisal, J. Clin. Endocrinol. Metab. 88 (2003) 4525–4532, https://doi.org/10.1210/jc.2003-030636.
[28] R.M. Krauss, Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk, Curr. Opin. Lipidol. 5 (1994) 339–349.
[29] M.A. Austin, M.C. King, K.M. Vranizan, R.M. Krauss, Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk, Circulation 82 (1990) 495–506, https://doi.org/10.1161/01.CIR.82.2.495.
[30] M.T. Hamilton, E. Areiqat, D.G. Hamilton, L. Bey, Plasma triglyceride metabolism in humans and rats during aging and physical inactivity, Int. J. Sport Nutr. Exerc. Metabol. 11 (2001) S97–S102, https://doi.org/10.1123/ijsnem.11.s1.s97.
[31] B.G. Nordestgaard, A test in context: lipid profile, fasting versus nonfasting, J. Am. Coll. Cardiol. 70 (2017) 1637–1646, https://doi.org/10.1016/j.jacc.2017.08.006.
[32] B.G. Nordestgaard, A. Varbo, Triglycerides and cardiovascular disease, Lancet 384 (2014) 626–635, https://doi.org/10.1016/S0140-6736(14)61177-6.
[33] A.B. Jørgensen, R. Frikke-Schmidt, A.S. West, P. Grande, B.G. Nordestgaard, et al., Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction, Eur. Heart J. 34 (2013) 1826–1833, https://doi.org/10.1093/eurheartj/ehs431.
[34] S.S. Martin, R.S. Blumenthal, M. Miller, LDL cholesterol: the lower the better, Med. Clin. North Am. 96 (2012) 13–26, https://doi.org/10.1016/j.mcna.2012.01.009.
[35] R. Pechlaner, S. Tsimikas, X. Yin, P. Willeit, F. Baig, et al., Very-low-density lipoprotein–associated apolipoproteins predict cardiovascular events and are lowered by inhibition of APOC-III, J. Am. Coll. Cardiol. 69 (2017) 789–800, https://doi.org/10.1016/j.jacc.2016.11.065.
[36] A. Varbo, M. Benn, A. Tybjærg-Hansen, A.B. Jørgensen, R. Frikke-Schmidt, et al., Remnant cholesterol as a causal risk factor for ischemic heart disease, J. Am. Coll. Cardiol. 61 (2013) 427–436, https://doi.org/10.1016/j.jacc.2012.08.1026.
[37] E.J. Parks, R.M. Krauss, M.P. Christiansen, R.A. Neese, M.K. Hellerstein, Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance, J. Clin. Invest. 104 (1999) 1087–1096.

[38] A.S. Wani, Zafirah, S. Rashid, H.M. Tantray, Carotid intima-media thickness as a marker for assessing the severity of coronary artery disease on coronary angiography, Int. J. Res. Med. Sci. 6 (2018) 1283–1287, https://doi.org/10.18203/2320-6012.ijrms20181283.

[39] A.H. Slyper, H. Rosenberg, A. Kabra, M.J. Weiss, B. Blech, et al., Early atherogenesis and visceral fat in obese adolescents, Int. J. Obes. 38 (2005) 954–958, https://doi.org/10.1038/ijo.2014.11, 2014.

[40] A. Rohatgi, A. Khera, J.D. Berry, E.G. Givens, C.R. Ayers, et al., HDL cholesterol efflux capacity and incident cardiovascular events, N. Engl. J. Med. 371 (2014) 2383–2393, https://doi.org/10.1056/NEJMoa1409065.

[41] R.K. Mutharasan, C.S. Thaxton, J. Berry, M.L. Daviglus, C. Yuan, et al., HDL efflux capacity, HDL particle size, and high-risk carotid atherosclerosis in a cohort of asymptomatic older adults: the Chicago Healthy Aging Study, J. Lipid Res. 58 (2017) 600–606, https://doi.org/10.1194/jlr.P069039.

[42] M.-R. Taskinen, J. Borén, New insights into the pathophysiology of dyslipidemia in type 2 diabetes, Atherosclerosis 239 (2015) 483–495, https://doi.org/10.1016/j.atherosclerosis.2015.01.039.

[43] A. Varbo, B.G. Nordestgaard, Commentary: Triglycerides or HDL cholesterol in cardiovascular disease—which is the true culprit?, Int. J. Epidemiol.. (n.d). https://doi.org/10.1093/ije/dyy292.

[44] A.L.M. Eikendal, K.A. Groenewegen, M.L. Bots, S.A.E. Peters, C.S.P.M. Uiterwaal, et al., Relation between adolescent cardiovascular risk factors and carotid intima-media echogenicity in healthy young adults: the atherosclerosis risk in young adults (ARYA) study, J. Am. Heart Assoc. 5 (2016), https://doi.org/10.1161/JAHA.115.002941.

[45] N. Brix, A. Ernst, L.L.B. Lauridsen, E. Parner, H. Støvring, et al., Timing of puberty in boys and girls: a population-based study, Paediatr. Perinat. Epidemiol. 33 (2019) 70–78, https://doi.org/10.1111/ppe.12507.

[46] I. Volanen, M.J. Järvisalo, R. Vainionpää, M. Arffman, K. Kallio, et al., Increased aortic intima-media thickness in 11-year-old healthy children with persistent Chlamydia pneumoniae seropositivity, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 649–655, https://doi.org/10.1161/01.ATV.0000202664.76816.bb.

[47] B.G. Nordestgaard, A. Langsted, S. Mora, G. Kolovou, H. Baum, et al., Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine, Eur. Heart J. 37 (2016) 1944–1958, https://doi.org/10.1093/eurheartj/ehw152.

[48] C. Stegemann, R. Pechlaner, P. Willeit, S.R. Langley, M. Mangino, et al., Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study, Circulation 129 (2014) 1821–1831, https://doi.org/10.1161/CIRCULATIONAHA.113.002500.

[49] H.O. Santos, C.P. Earnest, G.M. Tinsley, L.F.M. Izidoro, R.C.O. Macedo, Small dense low-density lipoprotein-cholesterol (sdLDL-C): analysis, effects on cardiovascular endpoints and dietary strategies, Prog. Cardiovasc. Dis. 63 (2020) 503–509, https://doi.org/10.1016/j.pcad.2020.04.009.