Cross-sectional and prospective associations between aerobic fitness and lipoprotein particle profile in a cohort of Norwegian schoolchildren

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ABSTRACT

Background and aims: The associations between aerobic fitness and traditional measures of lipid metabolism in children are uncertain. We investigated whether higher levels of aerobic fitness benefit lipoprotein metabolism by exploring associations with a comprehensive lipoprotein particle profile.

Methods: In our prospective cohort study, we used targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to profile 57 measures of lipoprotein metabolism from fasting serum samples of 858 fifth-grade Norwegian schoolchildren (49.0% girls; mean age 10.0 years). Aerobic fitness was measured using an intermittent shuttle run aerobic fitness test. We used multiple linear regression adjusted for potential confounders to examine cross-sectional and prospective associations between aerobic fitness and lipoprotein particle profile.

Results: Higher levels of aerobic fitness were associated with a favourable lipoprotein particle profile in the cross-sectional analysis, which included inverse associations with all measures of very low-density lipoprotein (VLDL) particles (e.g., −0.06 mmol·L^{-1} or −0.23 SD units; 95% CI = −0.31, −0.16 for VLDL cholesterol concentration). In the prospective analysis, the favourable pattern of associations persisted, though the individual associations tended to be more consistent with those of the cross-sectional analysis for the VLDL subclass measures compared to the low-density lipoproteins and high-density lipoproteins. Adjustment for adiposity attenuated the associations in both cross-sectional and prospective models. Nevertheless, an independent effect of aerobic fitness remained for some measures.

Conclusions: Improving children's aerobic fitness levels should benefit lipoprotein metabolism, though a concomitant reduction in adiposity would likely potentiate this effect.

1. Introduction

Higher levels of aerobic fitness in young adulthood are beneficially associated with a number of health outcomes in later life, including all-cause mortality and cardiovascular disease (CVD) [1]. In children, low aerobic fitness is strongly associated with clustered cardiometabolic risk [2]. Clustered cardiometabolic risk factors typically include clinical measures of lipid metabolism, such as blood triglycerides concentration,

dependent on the particular risk construct applied in studies. However, associations of aerobic fitness with individual measures of lipid metabolism are uncertain [3]. It is also unclear whether these associations are independent of adiposity or physical activity. Furthermore, the traditional clinical markers are limited in that they quantify a certain few lipoprotein lipid concentrations and provide no information on particle size or particle number, the latter of which is increasingly recognised as potentially the primary causative factor in atherogenesis, not the lipid

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load [4]. Nor can these measures demonstrate divergent directions of associations among lipoprotein subclasses, such as those observed for high-density lipoprotein (HDL) subclass concentrations with long-term leisure-time physical activity, adiposity, and risk of ischaemic stroke [5–7]. By providing a more nuanced description, metabolic profiling of the lipidome—lipidomics—can reveal more of the complexity of biological processes and interactions with environmental and lifestyle exposures, hence generate novel insights and hypotheses.

Our aim was to examine the cross-sectional and prospective associations between aerobic fitness and a comprehensive lipoprotein particle profile across the school year using targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy in a large cohort of Norwegian schoolchildren. As secondary aims, we examined potential confounding by adiposity and whether the associations were independent of moderate- to vigorous-intensity physical activity (MVPA).

2. Materials and methods

Additional methods are reported in the Supplementary Material.

2.1. Study population

We analysed data from the Active Smarter Kids (ASK) study. The ASK study was a cluster randomised controlled trial that investigated the effect of a seven-month school-based physical activity intervention on academic performance of fifth-grade schoolchildren in Sogn and Fjordane county, western Norway (https://clinicaltrials.gov, #NCT02132494) [8]. Sixty schools (1202 children) were approached, 57 of which agreed to participate (1129 children). Baseline testing of eligible children took place in 2014; follow-up testing eight months later. No differences were observed for levels of objectively measured physical activity or sedentary time between the intervention or control schools at either baseline or follow-up, hence we pooled the entire ASK study sample as one cohort for the present study [8]. The Regional Committee for Medical Research Ethics approved the study protocol. Written consent was obtained from each child's parent(s) or legal guardian(s) and from school authorities prior to testing. Procedures and methods abide by the World Medical Association's Declaration of Helsinki [9].

2.2. Assessment of aerobic fitness

Aerobic fitness was measured using the Andersen aerobic fitness test—an intermittent shuttle run test—performed according to standard procedures [10]. Study research assistants recorded the total distance (m) covered for each child. The Andersen test has been shown to be valid for estimating aerobic fitness at the group level in a cohort of similarly aged children and is used as a proxy for peak VO$_2$ [10]. Distance run was used as the main exposure rather than applying a prediction equation to estimate peak VO$_2$.

2.3. Assessment of lipoprotein measures

Overnight fasting blood samples for each child were drawn between 0800 and 1000 by a trained nurse or phlebotomist. Typically, these samples were taken within one week, and no more than one month, of the date of fitness testing.

Nuclear magnetic resonance spectra were recorded on a Bruker Avance III 600 MHz spectrometer, equipped with a QCI CryoProbe and automated sample changer (Bruker BioSpin GmbH, Karlsruhe, Germany). Spectral regions quantitatively associated to lipoprotein concentrations were selected as explanatory variables to partial least squares (PLS) modelling, performed for 106 randomly selected serum samples analysed by both ^{1}H NMR and high-performance liquid chromatography (HPLC) [11]. A Monte Carlo resampling approach was used to calculate individual PLS models with optimal prediction ability for

the HPLC data [12]. Lipoprotein particle numbers for all samples were subsequently predicted from these models, and the 20 lipoprotein subclasses were reduced to 15 [13]. Given the elution of lipid-poor pre-β$_1$ HDLs, only the HDL6 subclass measure was used when calculating the particle number for the HDL VS subclass [14]. Lipoprotein measures available for subsequent analysis comprised: total serum cholesterol concentration; total triglycerides concentration; non-HDL cholesterol concentration (calculated by subtracting HDL cholesterol concentration from the total cholesterol concentration); particle number, cholesterol concentration and triglycerides concentration of 15 lipoprotein subclasses; and average particle diameter of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and HDL particles. Though intact chylomicron particles cannot be distinguished from the largest VLDL particles using NMR spectroscopy, the nomenclature from the HPLC method, which does distinguish the two, was retained. For consistency, the chylomicron subclass was not incorporated when calculating measures of the VLDL class: VLDL cholesterol concentration, VLDL triglycerides concentration, or average VLDL particle size. However, given that the blood samples were drawn subsequent to an overnight fast, it is unlikely that these particles are of intestinal origin and should be considered very large VLDLs as opposed to chylomicrons [15].

2.4. Moderate- to vigorous-intensity physical activity

The children wore triaxial accelerometers (ActiGraph GT3X+, Acti-Graph LLC, Pensacola, FL) positioned on their right hip for seven consecutive days, but not during sleep or water-based activities. A valid day was considered as ≥480 min of monitor wear time between 0600 and 0000. Non-wear time was defined as ≥20 min of zero counts [16]. The accelerometer data were processed using commercially available Kinesoft software (version 3.3.80, KineSoft, Loughborough, United Kingdom) and 10-s epochs. Moderate- to vigorous-intensity physical activity was classified using the Evenson cut points of count data, which included all activity above the moderate-intensity physical activity (MPA) cut point (≥2296 counts·min^{-1}) [17,18].

2.5. Anthropometrics

Height was measured to the nearest 0.1 cm using a portable stadiometer, shoes removed, facing forwards (Seca 217, SECA GmbH, Hamburg, Germany); weight to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany). Body mass index (BMI) was calculated as weight divided by the square of height (kg·m^{-2}). Waist circumference was measured twice to the nearest 0.1 cm between the lower rib and iliac crest using a measuring tape (Seca 201, SECA GmbH, Hamburg, Germany), the child having gently exhaled. A third measurement was taken if the two differed by more than 1.0 cm. The mean of the two measurements with the least difference was used for analysis. Proportions of girls and boys classified as either overweight or obese were calculated using the International Obesity Task Force's (IOTF) sex-specific BMI cut-offs [19]. The children's ages at the time of testing were rounded down to the nearest half-year to provide conservative prevalence estimates.

2.6. Sexual maturity

The children self-reported their sexual maturity with reference to a standard set of colour images with accompanying descriptions corresponding to the Tanner staging method [20]. Low frequencies were recorded in Tanner categories 3, 4, and 5 (n = 66, 5, and 2, respectively of 1081 children with valid data at baseline), hence they were collapsed to one category for analysis (≥3).

2.7. Socioeconomic status

Socioeconomic status (SES) was quantified as the highest level of

educational attainment of either the child's mother, father, or guardian, whichever was the higher. Parent(s) or guardian(s) completed a custom self-report questionnaire, selecting their level of educational attainment as one of six categories. Of the six, low frequencies were recorded in the lower four categories ($n = 4, 15, 193,$ and 137 for categories 1–4, respectively of 1069 children with valid data), so were combined into one category "Upper secondary school" for analysis.

2.8. Statistical approach

We examined cross-sectional associations between aerobic fitness and 57 lipoprotein variables measured at baseline using separate linear regression models. Each model was adjusted for sex and parent's/guardian's education, and the baseline values of age and sexual maturity. To examine prospective associations, we regressed the 57 lipoprotein variables measured at follow-up on aerobic fitness measured at baseline. In addition to the covariates in the cross-sectional models, the baseline value of each respective lipoprotein variable was included.

To investigate potential confounding by adiposity on the associations between aerobic fitness and the lipoprotein measures, we first included baseline waist circumference in the cross-sectional and prospective aerobic fitness models. To compare the magnitudes of main effects of aerobic fitness and waist circumference, cross-sectional and prospective associations between waist circumference and the lipoprotein particle profile were examined, adjusting for the same covariates as in the aerobic fitness models. We also examined the cross-sectional and prospective associations between waist circumference and aerobic fitness, adjusting for the same covariates as in the aerobic fitness models, and additionally for aerobic fitness at baseline in the prospective model. Lastly, to assess whether the associations between aerobic fitness and the lipoprotein measures were independent of MVPA, we repeated each analysis additionally including baseline average daily MVPA as a covariate.

Aerobic fitness and all lipoprotein variables were converted to z-scores (mean $= 0.0$; SD $= 1.0$). Hence, regression coefficients represent the SD unit change in lipoprotein measure for a 1 SD increase in aerobic fitness. When examined as the primary exposure, the z-score of waist circumference was used. To account for potential within-cluster correlation and to obviate the need to transform skewed outcome variables, cluster and heteroscedasticity robust standard errors were calculated, clustered on school. To estimate the effective number of independent tests for multiple testing correction, principal component analysis (PCA) was used. The rationale for this method has been described previously and applied in a number of metabolic profile studies [21–23]. Five principal components explained >95% of the variance. Hence, the Bonferroni-corrected alpha threshold for assessing associations was $0.05/5 = 0.01$ ($p < 0.01$). For each outcome, the coefficients were reported in both absolute units and standardised units. The 95% confidence intervals (CIs) were given in standardised units. For each analysis, the sample comprised those children with valid data for each variable included in the respective regression model and hence the size varies.

We performed all statistical analyses in R version 3.6.3 [24]. In addition to base R functions, we used a variety of packages within the **tidyverse** (1.3.0) suite for data manipulation. We performed the linear regression analysis using the **estimatr** (0.22.0) package, specifically the **lm_robust** function. We plotted the results with **ggplot2** (3.3.0) and the custom visualisation functions **geom_stripes** and **facet_col** available in the **ggforestplot** (0.0.2) and **ggforce** (0.3.1) packages, respectively.

3. Results

3.1. Sample characteristics

There were 858 children (49.0% girls) with complete data for the main cross-sectional analyses of aerobic fitness (Supplementary Fig. 1). Those excluded children ($n = 271$) were similar to those with complete

data for the measured variables (Table 1). Of the 858 children included, 847 had valid data for MVPA recorded at baseline. Children with valid accelerometer data typically recorded higher average daily MVPA than those without. The average interval between baseline and follow-up testing was 34.3 weeks. Mean (SD) baseline values for the 57 lipoprotein measures are given in Supplementary Table 1.

Table 1
Characteristics of included children from the ASK study cohort.

Characteristic	Analytical sample ($n = 858$)		Excluded cases ($n = 271$)		Difference
	n (%[a])	Mean (SD)	Data available – n (%[a])	Mean (SD)	p value[b]
Age (years)		10.0 (0.3)	219	10.0 (0.3)	0.252
Sex					
Girls	420 (49.0)		121 (44.6)		0.217
Boys	438 (51.0)		150 (55.4)		
Anthropometry					
Height (m)		142.9 (6.8)	238	142.2 (6.7)	0.183
Weight (kg)		37.0 (8.1)	237	37.1 (8.1)	0.888
Body mass index (kg·m^{-2})		18.0 (3.0)	237	18.2 (3.1)	0.333
≥25	207 (24.1)		52 (21.9)		0.349
≥30	40 (4.7)		12 (5.1)		
Waist circumference (cm)		61.9 (7.4)	236	62.3 (7.6)	0.456
Parents' education[c]					
Upper secondary school	284 (33.1)		65 (30.8)		0.524
<4 years college/university	261 (30.4)		59 (28.0)		
≥4 years college/university	313 (36.5)		87 (41.2)		
Tanner stage[d]					
Stage 1	470 (54.8)		111 (49.8)		0.182
Stage 2	331 (38.6)		96 (43.0)		
Stage ≥3	57 (6.6)		16 (7.2)		
Andersen test (m)		897.8 (102.6)	78	886.1 (101.7)	0.337
MVPA (min·d^{-1})	847	76.5 (26.4)	213	68.9 (25.5)	<0.001
Lipid profile (baseline)					
TC (mmol·L^{-1})		4.5 (0.7)	150	4.4 (0.6)	0.390
LDL-C (mmol·L^{-1})		2.5 (0.6)	150	2.5 (0.6)	0.889
HDL-C (mmol·L^{-1})		1.6 (0.3)	150	1.6 (0.3)	0.167
TG (mmol·L^{-1})[e]		0.7 [0.5, 0.9]	150	0.7 [0.5, 0.9]	0.696[f]

ASK = Active Smarter Kids; BMI = body mass index; HDL-C = high-density lipoprotein; LDL-C = low-density lipoprotein cholesterol; MVPA = moderate- to vigorous-intensity physical activity; TC = total cholesterol; TG = triglycerides.

[a] Proportion of total n with data available for categorical variables.

[b] Continuous characteristics compared using linear regression, categorical characteristics using binomial logistic regression.

[c] For comparison, dichotomised to 'Upper secondary school' and 'Higher than upper secondary school'.

[d] For comparison, dichotomised to Tanner stage 1 and Tanner stage ≥2.

[e] Median [IQR].

[f] Comparison performed using log(TG).

3.2. Cross-sectional associations

Aerobic fitness was associated with all but one of the 57 lipoprotein measures analysed (Fig. 1). For a 1 SD increment in distance run for the Andersen test (103 m), there were inverse associations with almost all measures of apolipoprotein B-containing lipoproteins, including subclass particle numbers, and cholesterol and triglycerides concentrations (e.g., -2.61×10^{-1} nmol·L^{-1} or –0.21 SD; 95% CI = −0.30, −0.13; $p <$ 0.001 for VLDL L1 subclass particle number). The association with the average diameter of VLDL particles was also inverse, but positive with the average diameters of LDL and HDL particles. For HDL measures, the associations tended to differ dependent on subclass. Those with particle numbers, cholesterol and triglycerides concentrations were consistently inverse for the two smallest HDL subclasses. Higher aerobic fitness was positively associated with the particle numbers and cholesterol concentrations of the three largest HDL subclasses, and the effect sizes

tended to be larger than with those of the smaller subclasses. The associations were heterogeneous for the triglycerides concentrations of these three subclasses, being inverse for HDL M, positive for HDL L, and negligible for HDL VL. Regarding the more traditional lipid measures, higher aerobic fitness was inversely associated with total, non-HDL cholesterol, and LDL cholesterol concentrations, and total triglycerides concentration, but positively associated with HDL cholesterol concentration.

Having additionally included waist circumference as a covariate in the cross-sectional models, the effect sizes were attenuated for all but one lipoprotein measure (Fig. 2). The degree of attenuation varied between lipoprotein classes, within classes, and between different measures within the same subclass, but was more pronounced in the HDL subclasses.
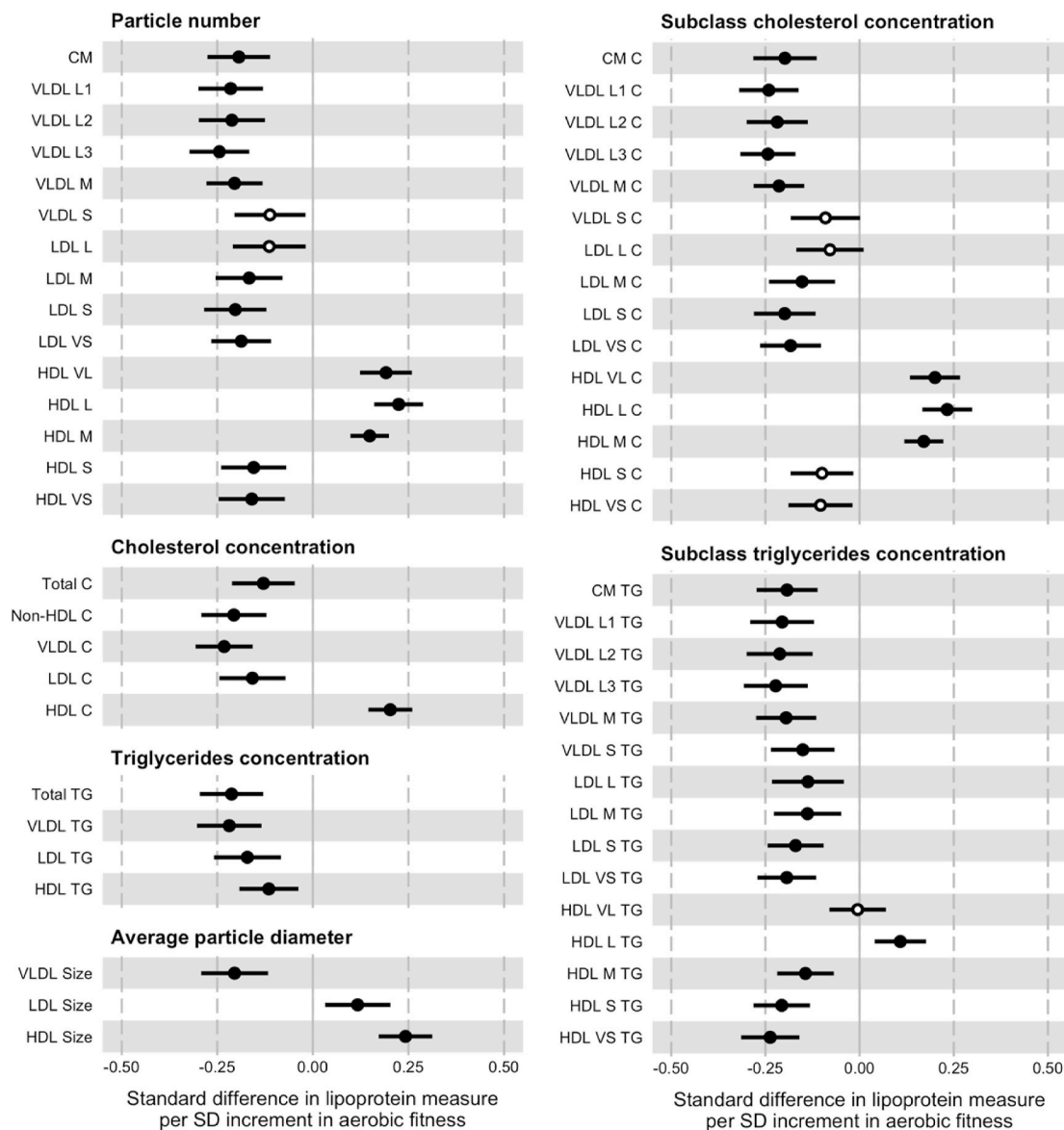

Fig. 1. Cross-sectional associations of aerobic fitness with 57 lipoprotein measures.
The associations were adjusted for age, parent's/guardian's education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in aerobic fitness. Filled circles are $p <$ 0.01. Error bars are 95% CIs. CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; SES = socioeconomic status; VLDL = very low-density lipoprotein; –C = cholesterol; -L = large; -M = medium; –S = small; -TG = triglycerides; -VL = very large; -VS = very small.
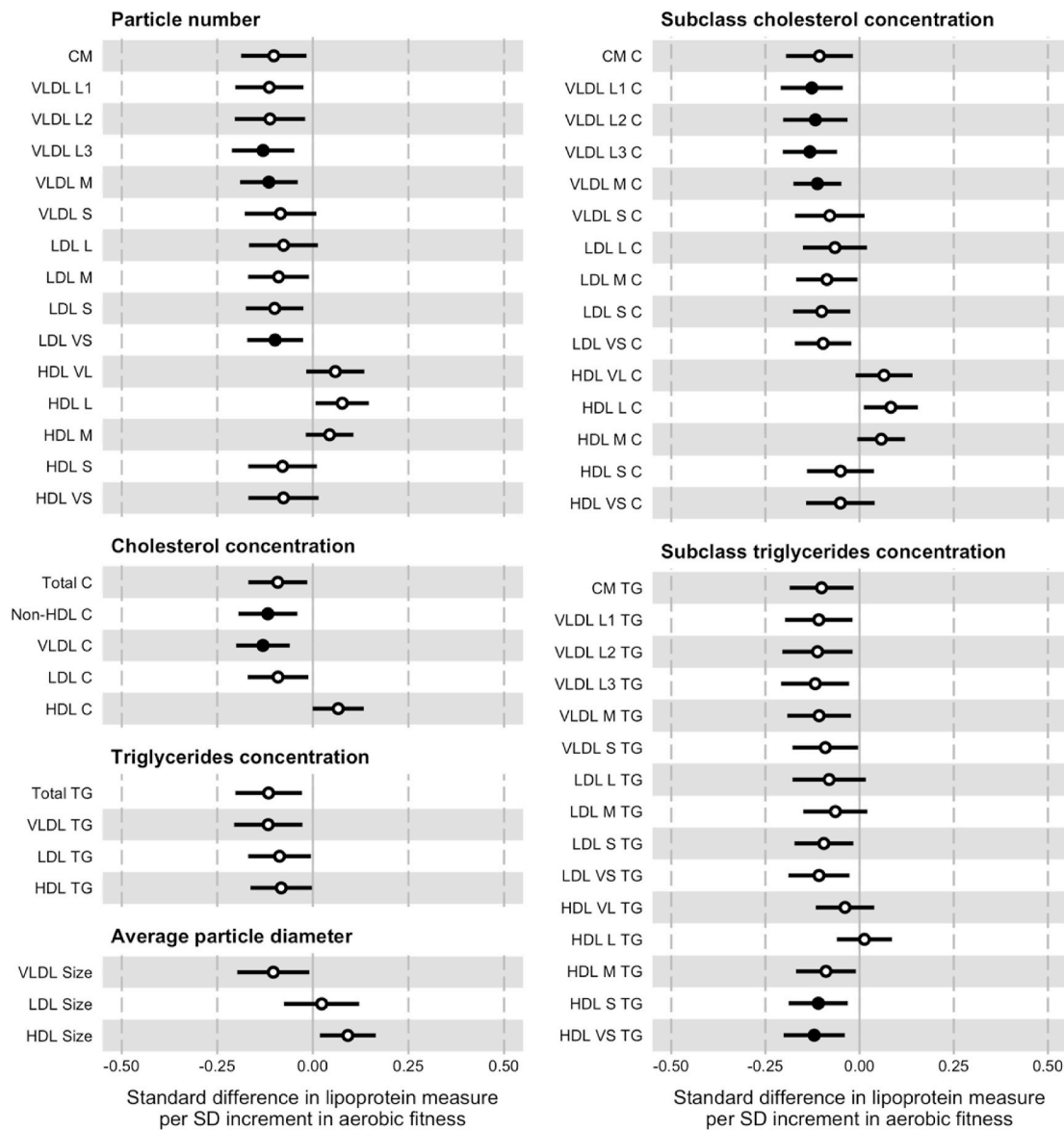
Fig. 2. Cross-sectional associations of aerobic fitness with 57 lipoprotein measures, adjusted for waist circumference.
The associations were adjusted for age, parent's/guardian's education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in aerobic fitness. Filled circles are $p < 0.01$. Error bars are 95% CIs. CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; SES = socioeconomic status; VLDL = very low-density lipoprotein; –C = cholesterol; -L = large; -M = medium; –S = small; -TG = triglycerides; -VL = very large; -VS = very small.

3.3. Prospective associations

The directions of associations remained the same as those in the cross-sectional analysis (Fig. 3). Effect sizes for all but one were attenuated, the degree of which varied considerably between individual measures. Comparing the prospective and cross-sectional analyses, the associations between a 1 SD increment in distance run for the Andersen test (102 m) with the VLDL subclasses were typically more consistent than with the LDL and HDL measures (e.g., -1.73×10^{-1} nmol·L^{-1} or –0.17 SD; 95% CI = $-0.26, -0.08$; $p < 0.001$ for VLDL L1 subclass particle number). The associations with subclass triglycerides concentrations tended to be more consistent compared to particle numbers or cholesterol concentrations for all lipoprotein classes. The attenuation of the association with the average diameter of HDL particles was more

marked than for the VLDL or LDL particles. Regarding the more traditional lipid measures, the association with total triglycerides concentration was more consistent than any measure of cholesterol concentration.

The pattern of associations remained broadly similar having included waist circumference as an additional covariate in the prospective models (Fig. 4). The effect sizes were generally more modest than in the other models but tended to be greater for the larger apolipoprotein B-containing particles (e.g., -7.74×10^{-2} nmol·L^{-1} or –0.08 SD; 95% CI = $-0.15, 0.00$; $p = 0.06$ for VLDL L1 subclass particle number). The direction of association changed with some measures of the larger HDL subclasses and also average HDL particle diameter, though the effect sizes were small both before and after including waist circumference in the models.
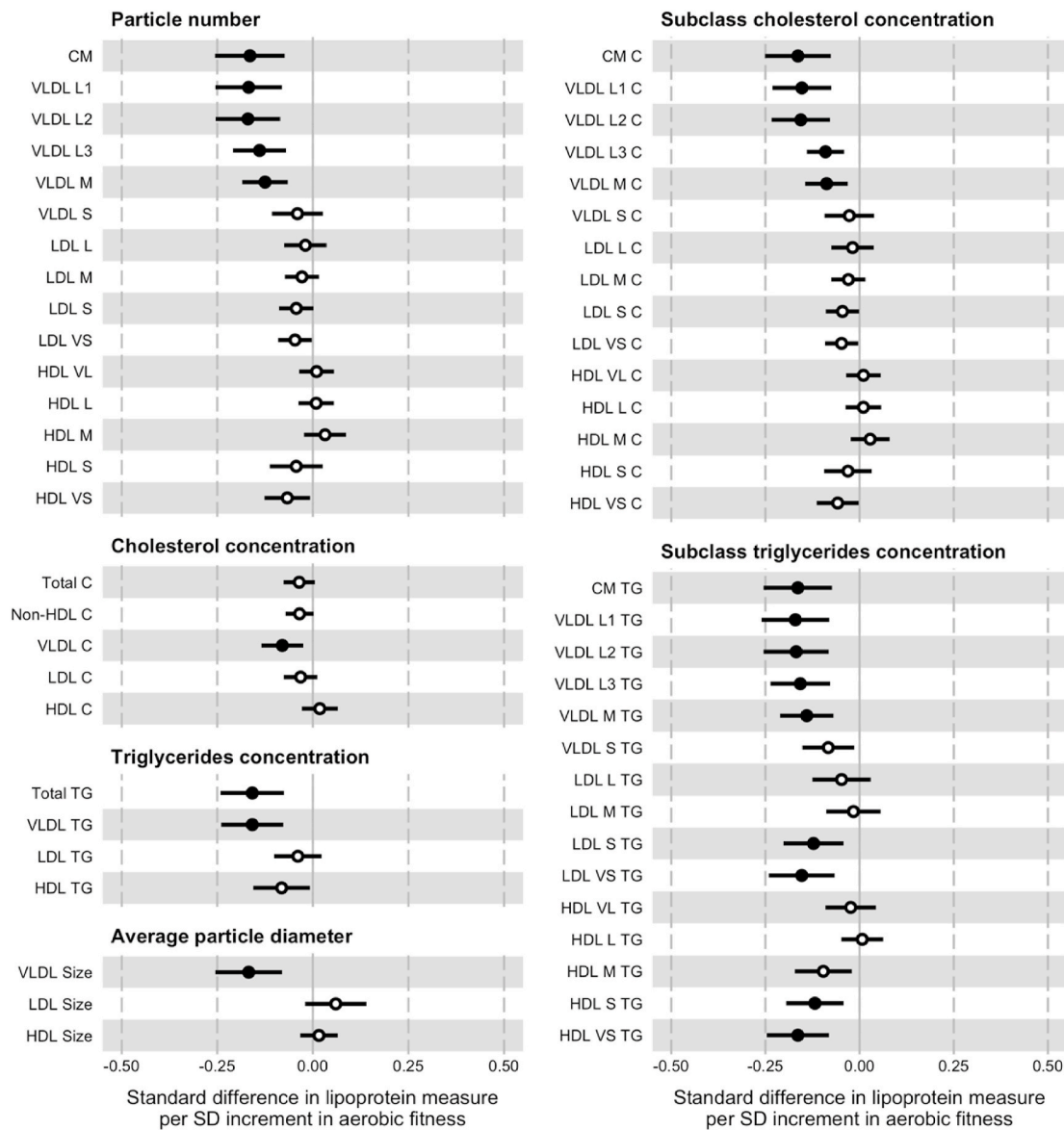
Fig. 3. Prospective associations of aerobic fitness with 57 lipoprotein measures.
The associations were adjusted for baseline values of age, lipoprotein measure, parent's/guardian's education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in aerobic fitness. Filled circles are $p < 0.01$. Error bars are 95% CIs. CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; SES = socioeconomic status; VLDL = very low-density lipoprotein; –C = cholesterol; -L = large; -M = medium; –S = small; -TG = triglycerides; -VL = very large; -VS = very small.

3.4. Supplementary results

In the cross-sectional analysis of waist circumference main effects, the directions of associations were the opposite to those of aerobic fitness for all but one of the 57 lipoprotein measures, though the overall pattern of associations was broadly similar (Supplementary Fig. 2). The main effects of a 1 SD increase in waist circumference (7.5 cm) were larger than those of aerobic fitness for all but two of the lipoprotein measures (e.g., 3.56×10^{-1} nmol·L^{-1} or 0.29 SD; 95% CI = 0.21, 0.38; $p < 0.001$ for VLDL L1 subclass particle number). Similarly, in the prospective analysis of waist circumference, the directions of associations were the opposite to those of the aerobic fitness analysis for all but one lipoprotein measure (Supplementary Fig. 3). The effect sizes were larger in the waist circumference model for all but three measures (e.g., 2.67×10^{-1} nmol·L^{-1} or 0.26 SD; 95% CI = 0.15, 0.36; $p < 0.001$ for VLDL L1 subclass particle number).

A 1 SD increment in waist circumference (7.4 cm) was inversely associated with aerobic fitness in both cross-sectional (-40 m or -0.39 SD units; 95% CI = -0.44, -0.34; $p < 0.001$) and prospective models (-17 m or -0.17 SD units; 95% CI = -0.22, -0.12; $p < 0.001$).

Comparing the results of aerobic fitness adjusted/not adjusted for average daily MVPA, the patterns of associations were similar and the directions of individual associations consistent for all but one of the 57 lipoprotein measures in both the cross-sectional and prospective analyses (Supplementary Figs. 4 and 5). Having adjusted for MVPA, the effect sizes increased for 21 of the lipoprotein measures in the cross-sectional analysis—predominantly those of the VLDL S and LDL subclasses—and decreased for 36 (e.g., -2.17×10^{-1} nmol·L^{-1} or -0.18 SD; 95% CI -0.27, -0.09; $p < 0.001$ for VLDL L1 subclass particle number), though the absolute differences tended to be small. In the prospective analysis, the effect of adjusting for MVPA was more consistent, the associations being attenuated for 52 of the 57 individual measures
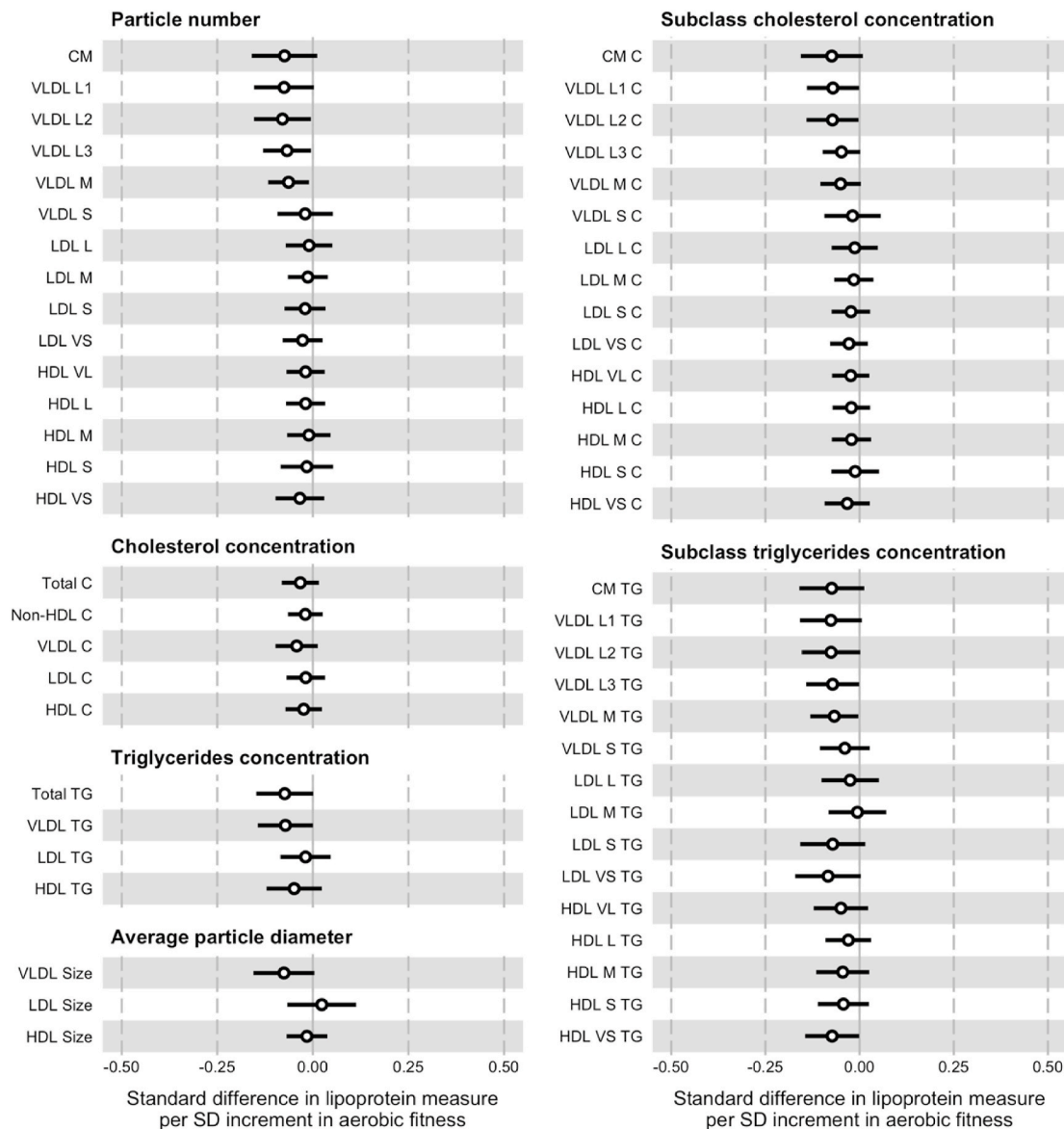
Fig. 4. Prospective associations of aerobic fitness with 57 lipoprotein measures, adjusted for waist circumference.
The associations were adjusted for baseline values of age, lipoprotein measure, parent's/guardian's education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in aerobic fitness. Filled circles are $p < 0.01$. Error bars are 95% CIs. CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; SES = socioeconomic status; VLDL = very low-density lipoprotein; –C = cholesterol; -L = large; -M = medium; –S = small; -TG = triglycerides; -VL = very large; -VS = very small.

compared to the model not adjusted for MVPA (e.g., -1.44×10^{-1} nmol·L^{-1} or –0.14 SD; 95% CI –0.23, –0.05, $p < 0.01$ for VLDL L1 subclass particle number). Again, the degree of attenuation tended to be small for each measure.

In both the cross-sectional and prospective analyses of aerobic fitness adjusted for waist circumference, the patterns and directions of associations were again similar having included average daily MVPA as a covariate (Supplementary Figs. 6 and 7). In the cross-sectional analysis, effect sizes increased for 19 of the individual lipoprotein measures—predominantly those of the VLDL S and LDL subclasses—and were attenuated for 38 measures. Comparing the prospective models, the effect of adjusting for MVPA was more consistent, associations being attenuated for 44 of the 57 measures. The degree of attenuation tended to be small in both the cross-sectional and prospective analyses.

The results of each analysis with outcome measures in absolute units are provided in Supplementary Tables 2–13.

4. Discussion

We used targeted metabolomics to investigate cross-sectional and prospective associations between aerobic fitness and lipoprotein particle profile. We found that aerobic fitness was associated with a favourable profile in our cohort of schoolchildren. The association magnitudes for all 57 lipoprotein measures tended to be small to moderate and diminished when examined prospectively, which indicates only modest influence of aerobic fitness on lipoprotein characteristics in this young, healthy cohort. Associations tended to be stronger with measures of VLDLs and particle triglycerides concentrations in prospective analyses, which may indicate a greater impact of aerobic fitness on these classes and lipids. The associations with the HDL subclasses differed greatly in magnitude and direction, seemingly dependent on particle size or lipid load. This may be a reflection of the substantial functional heterogeneity that exists between HDL particles [25].

Compared to aerobic fitness, the associations between a SD unit increment of waist circumference and almost all lipoprotein measures were stronger and likely explains why associations between aerobic fitness and these measures were attenuated following adjustment for waist circumference. In addition, waist circumference was inversely associated with aerobic fitness and therefore likely confounds the association between fitness and the lipoprotein profile. This may also reflect a lack of precision in our measure of aerobic fitness or the influence of body size on aerobic performance tests [26]. Still, adjustment for waist circumference as a covariate did not completely eliminate the associations and suggests that some influence of aerobic fitness is independent of adiposity.

Adjusting the aerobic fitness models for average daily MVPA had a small effect, indicating that the associations between aerobic fitness and lipoprotein measures are largely independent of MVPA, though a mechanism by which higher levels of fitness might affect lipoprotein metabolism independent of energy expenditure through physical activity is uncertain [27].

To our knowledge, this is the first study to investigate prospective associations between aerobic fitness and a comprehensive lipoprotein profile in children. A recent cross-sectional study in adults examined associations of the serum metabolome between high and low aerobic fitness groups and showed a favourable pattern of associations with lipoprotein measures in those who were more fit [28]. In supplementary analyses, the authors demonstrated that although the associations of aerobic fitness with a number of individual measures were partly independent of age and body fat percentage, its inclusion as a variable in the regression models accounted for little additional variance compared to body fat percentage and age alone. In contrast, a Mendelian randomisation analysis in young adults found that many of the associations between adiposity and lipoprotein particle measures are strong and likely causal [6]. Studies investigating physical activity in children and youth have shown that higher levels of physical activity are both favourably associated with a number of lipoprotein particle measures independent of adiposity and might also prevent excessive adiposity [23,29]. However, the associations between physical activity and adiposity are likely bidirectional and tend to be stronger when adiposity is modelled as the exposure [30]. Reducing adiposity should therefore be a priority, whether achieved through increased physical activity or other means, such as caloric restriction [31]. Not only would this benefit lipoprotein metabolism directly, but also through the independent effects of a reciprocal increase in physical activity, and any resulting increase in aerobic fitness [32].

Strengths of our study include the detailed measures of lipoprotein particle profile quantified using a targeted NMR metabolomics platform, which enabled the examination of a number of different lipoprotein attributes not measured in a standard lipid profile. The collection of blood samples at two time points permitted investigation of the temporal sequence of associations, which affords greater confidence in the resultant associations than cross-sectional studies. We had a relatively large sample size given the population and measures analysed, and high compliance of the children with fitness testing, blood sampling and accelerometry. The blood samples were drawn from the children whilst fasted and at a consistent time of day, limiting potential variability due to dietary intake and daily biological and behavioural rhythms. Device-based measures of physical activity, such as accelerometers, typically exhibit lower variability than self-report methods when compared to criterion measures and are less prone to the subjective biases of participants [33]. The Andersen test is both a valid and reliable tool for assessing aerobic fitness at group level, and hence appropriately applied in the present observational study [10].

There are also limitations with our study. Given the homogeneity of the children included in our sample, our results may not be generalisable to more diverse populations. However, the prevalence of overweight and obesity in our sample is comparable to those of similarly aged children from other European countries [34]. We followed the children

for just under one academic year and are therefore unable to describe how the associations between their aerobic fitness and metabolic traits develop over longer durations or into adolescence and adulthood. Importantly, though we have included repeat measures of lipoprotein particle profiles, they are static snapshots of dynamic processes so can only speculate as to any mechanistic explanations for these associations. The high compliance with testing permitted adjustment for a number of putative confounders, yet we cannot exclude residual confounding from other variables unmeasured. Pertinently, we lacked information on energy intake and dietary composition for our children. Field tests, such as the Andersen test, are performance-based and do not directly measure peak VO$_2$. Though these tests have obvious advantages in large population studies compared to laboratory testing and appear valid for estimating aerobic fitness at the group level, they may be influenced far more by anthropometric and psychological factors than laboratory measurements of peak VO$_2$ by indirect calorimetry [35]. However, previous studies on a smaller but similar cohort showed significantly greater impact of BMI on peak VO$_2$ than on performance in the Andersen test, and stronger associations with lipoprotein measures for the Andersen test than for peak VO$_2$ [36,37]. Still, these results should be interpreted carefully due to the relatively small sample size and different analytical approach used. Lastly, given that our study was exploratory, replication of our findings in larger populations with longer follow-up durations and greater metabolic variance are necessary to determine whether the associations between aerobic fitness and lipoprotein particle measures are causal and to evaluate any health effects.

4.1. Conclusion

In conclusion, higher levels of aerobic fitness are prospectively associated with a favourable lipoprotein particle profile in our cohort of healthy, Norwegian schoolchildren. Though adiposity attenuated most associations, independent associations with aerobic fitness remained for a number of the measures examined. This suggests that improving population fitness levels may have beneficial effects on lipoprotein metabolism, though a concomitant reduction in adiposity would likely be more effective.

Author contributions

PRJ, GKR, EA, JS-J, SAA, OMK, and UE contributed to the conception and design of the work. TR, GKR, EA, TFB, and TA contributed to data acquisition. PRJ, TR, TFB, TA, and OMK contributed to data analysis. PRJ, EA, JS-J, SAA, OMK, and UE contributed to interpretation of the results. PRJ and UE drafted the manuscript. All authors critically revised the manuscript, gave final approval, and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.02.002.

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