DISSERTATION FROM THE
NORWEGIAN SCHOOL OF
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Paul Remy Jones

The associations of physical activity, sedentary time, and aerobic fitness with lipoprotein particle profile in children

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Summary

Introduction: Metabolomics can elucidate the metabolites and pathways of human metabolism. Its application in epidemiological studies promises to improve our understanding of health and its connection to lifestyle behaviours, such as physical activity.

Purpose: I. To examine the cross-sectional and prospective associations of physical activity, sedentary time, and aerobic fitness with a detailed lipoprotein profile. **II.** To consider the theoretical effect on the lipoprotein profile of replacing time spent sedentary with an equal amount of time in moderate- to vigorous-intensity physical activity (MVPA). **III.** To determine whether daily time spent participating in MVPA moderates the prospective associations between aerobic fitness and lipoprotein subclass particle numbers. **IV.** To investigate the extent to which the associations of physical activity, sedentary time, and aerobic fitness with the lipoprotein profile are attenuated or confounded by adiposity.

Participants and methods: The information is taken from the Active Smarter Kids (ASK) study, which was a cluster randomised controlled trial of a school-based physical activity intervention conducted over one academic year in 2014–15. The participants were children in the fifth grade whom attended school in Sogn and Fjordane county, Norway. Of those children invited and eligible to participate, 1129 underwent baseline testing. The children were retested towards the end of the intervention period. Physical activity and sedentary time were measured using accelerometers, and aerobic fitness assessed as the distance run in the Andersen intermittent running test. The circulating lipoprotein profile was quantified using proton nuclear magnetic resonance spectroscopy. Waist circumference was used as a proxy for adiposity.

Main results: I. Higher levels of moderate-intensity physical activity (MPA) and vigorous-intensity physical activity (VPA) were associated with an apparently favourable lipoprotein

profile in both cross-sectional and prospective analyses. The most marked associations were with measures of very low-density lipoproteins (VLDLs). In the light-intensity physical activity (LPA) analyses, the associations were generally more modest. More time spent sedentary was associated with an apparently unfavourable profile. The associations were typically the inverse of those in the VPA analyses. The pattern of associations between aerobic fitness and the lipoprotein profile was very similar to that with VPA. **II.** Substituting 30 minutes of MVPA for an equal amount of sedentary time resulted in a lipoprotein profile comparable to that of the single activity MVPA analysis. **III.** MVPA appears to moderate the beneficial associations between aerobic fitness and the circulating numbers of larger VLDL particles in those children with lower aerobic fitness levels. **IV.** Adiposity attenuated many of the individual associations in most analyses. This effect was more pronounced in the aerobic fitness analyses.

Conclusions: Physical activity of at least moderate intensity appears beneficial for lipoprotein metabolism as a result of reductions in the number of circulating VLDL particles, possibly due to an acute exercise-induced energy deficit. Light-intensity physical activity seems of limited influence. The unfavourable associations of sedentary time are potentially attributable, at least in part, to less time spent physically active as opposed to a direct consequence of more time spent sedentary. Replacing sedentary time with MVPA theoretically ameliorates these detrimental associations. Though aerobic fitness appears of benefit, the associations are heavily influenced, possibly confounded, by adiposity levels. The associations of physical activity and sedentary time appear partly independent of adiposity. These results suggest that increasing PA levels in children could benefit lipoprotein metabolism, especially in less fit children. A concomitant reduction in sedentary time and adiposity would likely be synergistic.

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Natsai. Your love makes it all worthwhile.

London, July 2020

Paul Remy Jones

Abbreviations

^{1}H NMR	Proton nuclear magnetic resonance
20mSRT	20-m shuttle run test
AEE	Activity-induced energy expenditure
AI	Artificial intelligence
ApoB	Apolipoprotein B
ASCVD	Atherosclerotic cardiovascular disease
ASK	Active Smarter Kids
BEE	Basal energy expenditure
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
CVD	Cardiovascular disease
DLW	Doubly labelled water
EE	Energy expenditure
FDR	False discovery rate
FFA	Free fatty acid
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HMGCR	HMG-CoA reductase
HPLC	High-performance liquid chromatography
HR	Heart rate
HRM	Heart rate monitor
IDL	Intermediate-density lipoprotein

IMT	Intima-media thickness
IOTF	International Obesity Task Force
IQR	Interquartile range
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LPL	Lipoprotein lipase
LPA	Light-intensity physical activity
Lp(a)	Lipoprotein(a)
MET	Metabolic equivalent of task
MetS	Metabolic syndrome
MI	Myocardial infarction
MPA	Moderate-intensity physical activity
MVPA	Moderate- to vigorous-intensity physical activity
MS	Mass spectrometry
NCD	Non-communicable disease
NDET	Norwegian Directorate for Education and Training
NMR	Nuclear magnetic resonance
PA	Physical activity
PAD	Peripheral artery disease
PAL	Physical activity level
PE	Physical education
PLS	Partial least squares
PPL	Postprandial lipaemia
RCT	Reverse cholesterol transport

SD	Standard deviation
SES	Socioeconomic status
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TEE	Total energy expenditure
VLDL	Very low-density lipoprotein
VPA	Vigorous-intensity physical activity

List of papers

This thesis centres on four original research papers, denoted by Roman numerals:

I. Jones PR, Rajalahti T, Resaland GK, Aadland E, Steene-Johannessen J, Anderssen SA, Bathen TF, Andreassen T, Kvalheim OM, Ekelund U. Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis* 2019; 288: 186–93.

II. Jones PR, Rajalahti T, Resaland GK, Aadland E, Steene-Johannessen J, Anderssen SA, Bathen TF, Andreassen T, Kvalheim OM, Ekelund U. Prospective associations between aerobic fitness and lipoprotein subclasses in a cohort of Norwegian schoolchildren.

III. Jones PR, Rajalahti T, Resaland GK, Aadland E, Steene-Johannessen J, Anderssen SA, Bathen TF, Andreassen T, Kvalheim OM, Ekelund U. Associations of lipoprotein profile and objectively measured physical activity and sedentary time in schoolchildren: a prospective cohort study.

IV. Jones PR, Rajalahti T, Resaland GK, Aadland E, Steene-Johannessen J, Anderssen SA, Bathen TF, Andreassen T, Kvalheim OM, Ekelund U. Moderation of the association between aerobic fitness and lipoprotein subclass particle numbers by moderate- to vigorous-intensity physical activity: a prospective cohort study.

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1. Introduction

Globally, non-communicable diseases (NCDs) are the leading causes of mortality, accounting for three in every four deaths.[1] Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels and the leading cause of mortality worldwide, responsible for an estimated 17.8 million deaths each year.[1] A number of risk factors for the development of CVDs—and NCDs in general—are behavioural, such as smoking tobacco, and hence a large proportion of the global CVD burden is believed preventable through the avoidance or reduction of certain behaviours.[2] Indeed, one such behavioural risk factor, *physical inactivity*—a lack of sufficient physical activity (PA) to meet current recommendations—has been estimated as being responsible for more than 9% of premature mortality globally and up to 7.8% of the burden of coronary heart disease (CHD)—the most common CVD.[3,4]

That there are benefits of engagement with PA and health, however defined, is not new knowledge, with evidence of its prominence in the practices of a variety of cultures for millennia.[5] In more recent history, the pioneering work of Jerry Morris demonstrated the importance of PA for health and went a long way to founding the field of PA epidemiology. In his study of London Transport workers, he found that when compared to the more sedentary bus drivers, the more active bus conductors had a lower incidence of CHD, their CHD appeared at a later age, and the CHD that they did develop was less severe.[6] Research into the protective effects of PA has continued ever since, establishing associations with a number of diverse health outcomes and culminating in the development and publication of evidence-based national and global PA guidelines and recommendations for both adults and children to inform interventions and policy.[4,7]

Although the clinical complications of CVDs typically manifest in adulthood, atherosclerosis—the main pathological process that contributes to the atherosclerotic

cardiovascular diseases (ASCVDs): CHD, stroke, and peripheral artery disease (PAD)—begins in the first decade of life.[8] Furthermore, common cardiovascular risk factors measured in childhood and adolescence have been shown to predict the intima-media thickness (IMT) of the carotid arteries—a measure of sub-clinical atherosclerosis—in young adulthood and middle-age.[9] This prediction was independent of the same risk factors measured contemporaneous with the IMT.[10] There is contradictory evidence regarding whether the effects of certain risk factors, such as lipid levels and dyslipidaemia, are a result of lipid levels tracking into adulthood, or whether detrimental lipid levels in childhood cause permanent structural changes to the vasculature, which increase risk independent of adult levels.[11,12] Either way, that atherosclerosis begins early in life has prompted a shift towards primordial prevention—the avoidance or reduction of risk factors—of CVD, accompanied by a concomitant research focus on understanding the mechanisms through which healthy lifestyles and behaviours, such as PA, exert their beneficial cardiometabolic effects in children.[13]

1.1. Physical activity

1.1.1. Definition

The term *physical activity* refers to any bodily movement produced by skeletal muscles that expends energy.[14] It is a behaviour whose definition encompasses a broad spectrum of activities and consequent energy expenditures from swiping one's finger across the touch screen of their mobile phone to running a marathon. Though a complex behaviour, it is possible to measure certain dimensions of PA to better quantify and study it.[15] The *mode* of PA is the particular activity being performed, such as walking, swimming, or chopping wood. The *duration* refers to the amount of time spent undertaking a bout of PA within a given time frame, whereas the *frequency* is the number of bouts performed per time unit, and *intensity* is the rate of energy expenditure, which is an indicator of metabolic demand. The *volume* of PA is the duration

multiplied by the frequency and intensity. PA also takes place in a variety of contexts, *domains*, which can be categorised, such as being work-related, domestic, or performed for recreation.

1.1.2. Measurement

There are a variety of methods with which to measure PA. No one method can quantify all PA dimensions well and each has its own advantages and compromises dependent on the particular research question to be answered and practicalities associated with performing the research. For example, direct observation of an individual by a trained researcher can provide a wealth of contextual information but is less objective that many other methods and requires substantial financial and time commitments.

The *reliability* and *validity* of each measurement technique are important considerations. Reliability describes the consistency and stability of results produced when using a given technique, such as whether the same results will be obtained by two independent assessors (inter-rater reliability) or the agreement between repeated administration of a given test by a single individual (intra-rater reliability). Validity refers to whether a given measurement technique actually measures what it is purported to. It is also important that individual measurement techniques are *accurate*, in that the measurements they produce are close to the true value, and *precise*, whereby repeated measurements of the same thing are close to each other. Though a measurement technique could be all of these things, its use may not be feasible in all settings. Hence, a range of different techniques with which to measure PA exist and the most appropriate method(s) for a given research study is(are) usually dependent on a number of situation-specific considerations.

Criterion standards

Indirect calorimetry

Calorimetry is the measurement of heat transfer. In the study of human biology, energy is typically measured in kilocalories (kcal). One kcal is defined as the heat required to raise the temperature of 1 kg (1 L) of water by 1°C. Human energy expenditure (EE) can be measured using either *direct calorimetry*—measuring heat loss directly—or using *indirect calorimetry*—measuring the exchange of O_2 and CO_2 as a proxy for heat loss.[16] Direct calorimetry is usually performed in an insulated chamber in a laboratory, is technically difficult and expensive, and hence used much less commonly than indirect calorimetry.

Since human energy production relies on aerobic respiration, an individual's EE can be accurately determined by measuring their O_2 consumption. For every litre of O_2 consumed, ~5 kcal is utilised. Given the O_2, CO_2, and nitrogen concentrations in ambient air are known, the differences in O_2 and CO_2 between inspired and expired air and the respiratory rate can be used to calculate the amount of energy used for aerobic respiration.[16] Though indirect calorimetry can be performed in metabolic chambers, small metabolic carts with ventilated hoods are a more portable alternative which makes deployment in clinical settings and in the field possible.[17] However, as a technique to measure PA over a prolonged period in free-living conditions, it is currently too impractical and consequently alternative PA measurement techniques are used.

Doubly labelled water

Doubly labelled water is a radio-labelled isotope of water ($^2H_2\ ^{18}O$) administered orally. Over the following 1 to 4 weeks, both the deuterium (^2H) and ^{18}O are eliminated from the body. Since the ^2H is eliminated as water only, and the ^{18}O as either water or CO_2, the difference between the two elimination rates is proportional to CO_2 production and hence EE. Having also measured a participant's basal energy expenditure (BEE), PA can then be calculated as activity-

induced energy expenditure (AEE = 0.9 x total energy expenditure [TEE] – BEE). In this equation, diet-induced energy expenditure is assumed to be 10% of TEE (0.9 x TEE) in subjects consuming an average mixed diet and in energy balance.[18] An alternative method is to calculate PA as the physical activity level (PAL = TEE/BEE).

DLW is relatively simple to administer and can be used to measure EE in free-living conditions. It is also accurate to within 3 to 4% of indirect calorimetry and is therefore considered a criterion measure of PA in field settings.[19] However, a number of constraints limit the use of DLW in large-scale population studies, including the difficulty obtaining and expense of the isotopes. Furthermore, DLW only measures TEE and provides no information on PA duration, intensity, or frequency, which may have distinct effects on a given outcome compared to TEE.

Direct observation

Direct observation typically involves trained researchers assessing the PA of groups in specific but free-ranging settings, such as physical education (PE) classes and parks and coded into categories in real-time. A variety of different observation measures exist, and each has its own sampling and recording system.[19] Direct observation facilitates the concurrent assessment of a number of PA dimensions and the data obtained tends to be of high quality. Inter-rater reliability is high.[19] However, direct observation has a relatively high burden on the researcher, is costly, due to the need to train and deploy observers, and, if aware that they're being assessed, may evoke reactivity effects in the participants.[20] The infeasibility of observing and recording the PA of entire cohorts in all settings limits its application in large population studies.

Self-report methods

Broadly, techniques used to measure free-living PA can be dichotomised into those that are self-report and those that are device-based. Self-report methods are so-called because they require either an individual or a proxy to assess and report their PA. Diaries, logs,

questionnaires, and surveys have all been used in research to assess PA.[21] These techniques are cost effective, and some, such as questionnaires, relatively simple to employ in large population studies. Dependent on the particular self-report measure, both respondent and assessor burden can be low. They can also obtain information on domains of PA, which are otherwise difficult to assess with device-based measures such as accelerometers. Unfortunately, the reliability and validity of questionnaires tend to be poor.[22] For those self-report methods that produce more accurate and detailed assessments of PA, such as activity diaries, the burden of use can be high, which precludes their application in the study of certain populations including young children.[19] Other issues common, but not limited, to self-report methods are their proneness to certain biases, including participant exaggeration and social desirability bias, and their reliance on individuals being able to accurately recall and quantify their PA behaviour. For this reason, accurately measuring shorter durations/bouts of activity is difficult and likely inaccurate.

Device-based methods

Device-based methods involve the use of a wearable device. In recent decades, advances in technology have resulted in PA measurement devices that are relatively cheap, unobtrusive, and importantly, valid. As these devices have developed, their use in epidemiological studies has increased markedly. Two commonly used classes of devices are heart rate (HR) monitors and motion sensors.

Heart rate monitors

Heart rate monitors (HRMs) rely on the linear relationship between oxygen consumption ($\dot{V}O_2$) and HR when exercising.[23] Though this assumption is appropriate for moderate- to vigorous-intensity physical activity (MVPA), at lower intensities HR can be heavily influenced by factors not related to activity, such as emotional stress, environmental temperature, and caffeine.[15] One approach to overcome this limitation is to individually calibrate the linear

relationship and establish a HR threshold—the flex point (flex HR)—which discriminates between resting EE and AEE.[23] Still, there is substantial intra-individual variation using the flex HR method and although estimates can be improved with repeated calibration this is both time- and labour-intensive, which limits its use in large studies.[19]

Pedometers

Pedometers are motion sensors which can estimate number of steps taken by recording movement during the gait cycle.[15] Accuracy is better at faster walking speeds than slow. Their accuracy measuring steps is very high, with some models recording mean values within ±1% compared to direct observation.[24] Strong correlations between step frequency measured using pedometers and $\dot{V}O_2$ have been reported in controlled laboratory conditions in children.[25] However, EE tends to be underestimated in free-living conditions as pedometers cannot measure certain activities, such as cycling, nor other step-based activities that have higher energy demands, such as carrying weight. Though cheap and simple to use, their inability to accurately assess PA intensity, frequency, or duration, limits their applicability compared to accelerometers.

Accelerometers

Accelerometers are motion sensors that measure acceleration of the body during movement. Acceleration occurs in one of three planes; newer devices can capture all three. These accelerations—measured in units of acceleration due to gravity (g) and expressed in metres per second squared ($m \cdot s^{-1}$)—are sampled at frequencies greater that once per second and filtered to lower-resolution user-defined epochs, which commonly range from 5 to 60 seconds.[15,21] Accelerometer data is usually expressed in counts, to which PA intensity thresholds can be applied to derive the frequency and duration spent in sedentary, light, moderate, and vigorous intensities.[26]

Physical activity varies from day-to-day and between weekdays and weekend days. Therefore, assessment tends to occur over a number of consecutive days. Accelerometers are typically worn on the hip or lower back. For this reason, they are poor at capturing upper body movements and activities such as cycling.[21] They have to be removed for swimming and, similar to pedometers, underestimate EE when an individual is carrying weight or walking on an incline.[27] Though a number of accelerometers are commercially available, the data processed differently dependent on the device's manufacturer—and usually with proprietary algorithms—which has hindered comparisons between monitors and studies.[28] Despite their limitations, accelerometers are used widely and to good effect in large-scale epidemiology studies.

The accelerometers of one company, ActiGraph (ActiGraph LLC, Pensacola, FL), have been used in a number of studies of PA in children and adolescents, including those of this thesis.[29,30] Older models have compared well to criterion measures of EE dependent on mode of PA. A study of 10 to 14-year-old children reported a strong correlation between an activity monitor and EE and $\dot{V}O_2$ measured by indirect calorimetry whilst walking and running on a treadmill.[31] Correlation is diminished when the exercise protocols use more representative childhood activities, such as hopping or catching, but remains acceptable.[25] Though naturally lower under free-living conditions, activity counts were associated with both TEE and AEE assessed using DLW.[32] A recent investigation found the more modern GT3X+ model to have good inter-instrument reliability when measuring overall PA level, intensity-specific PA, and sedentary time in adults under free-living conditions.[33] In a sample of 9 to 11-year-old children from 12 countries, wearing the GT3X+ for seven consecutive days and having at least four days of valid data—at least 10 h of waking wear time, including one weekend day—was appropriate to achieve a reliability estimate of $\geq$0.8.[34] Though various sets of cut points were developed with

which to divide activity counts into different intensities, only one—the Evenson cut points—were found to provide acceptable classification accuracy for all four levels of PA intensity.[35]

1.2. Sedentary behaviour and sedentary time

1.2.1. Definition

Sedentary behaviour has been defined as *any waking behaviour characterized by an energy expenditure ≤1.5 METs* [metabolic equivalent of tasks] *while in a sitting or reclining posture*.[35] Sedentary behaviour is considered distinct from *sedentary time*, which refers to the time spent engaged in sedentary behaviours.[36] Explicit in its definition, sedentary behaviour is quantified according to the combination of posture, which can be inferred from the mode and/or domain, and activity intensity. Sedentary time additionally includes the duration. Though the distinction is evident, a consensus opinion on the definitions of both sedentary behaviour and sedentary time has only recently been reached.[36]

To compound confusion, within the research literature being sedentary has been considered synonymous with being physically inactive. Yet physical inactivity is defined as a level of PA insufficient to meet PA recommendations.[4] These definitions of sedentary behaviour and physical inactivity are not necessarily mutually exclusive and it is indeed possible to be highly sedentary whilst accumulating daily PA volumes above the physical inactivity threshold.[37] Hence, the terms sedentary and inactive should not be used interchangeably.

1.2.2. Measurement

Many of the methods used to measure PA are also used to measure sedentary behaviour or sedentary time.[38] One additional device of note is that of posture monitors, which can be used to classify time spent sitting or lying, standing, and stepping, and provides information on step number, cadence, and transitions between sitting and standing. Currently, research on validity

is limited, but they have been found valid and reliable measures of measuring step counts and more sensitive in reductions to sitting time than accelerometers in adults.[39,40]

1.3. Aerobic fitness

1.3.1. Definition

Aerobic fitness, or *cardiorespiratory fitness*, is a physiological measure of the ability to deliver oxygen to mitochondria during physical work.[41] Aerobic fitness reflects the integrated functioning of a number of organ systems—primarily respiratory, cardiovascular, and muscular—and is consequently considered indicative of overall health.

1.3.2. Measurement

For over half a century, maximum oxygen uptake ($\dot{V}O_{2max}$) has been considered the reference standard for assessing aerobic fitness.[42] Determining $\dot{V}O_{2max}$ requires an individual to exercise at progressively higher intensities until their $\dot{V}O_2$ no longer increases linearly, but plateaus.[43] However, in children exercising to near exhaustion, only a small minority record a $\dot{V}O_2$ plateau.[44] Consequently—though there is evidence to suggest that $\dot{V}O_{2max}$ is achieved in spite of not reaching a plateau—the criterion measure of aerobic fitness in children is peak oxygen uptake ($\dot{V}O_{2peak}$), obtained at volitional exhaustion.[45,46] This is routinely determined by running on a treadmill or cycling a cycle ergometer. The requirement for expensive and cumbersome equipment, and the burdensome process to perform the testing of $\dot{V}O_{2peak}$ is a hindrance to its measurement in epidemiology studies. In contrast, field tests—which include the 20-metre shuttle run test (20mSRT), distance/timed walk/run tests, and the Andersen test—are simpler to implement and facilitate assessment of aerobic fitness in large cohorts of children.[47,48] There is debate regarding how valid a measure of $\dot{V}O_{2peak}$ field tests are citing the undue influence of fat mass on outcomes, which should not, by definition, contribute to maximum oxygen

consumption.[49,50] Nonetheless, a recent meta-analysis reported the 20mSRT to have 'moderate' criterion validity for estimating aerobic fitness in children, whilst another study, also in children, found the Andersen test to be valid and reliable for estimating $\dot{V}O_{2peak}$ at the group-level.[51,52]

1.4. Lipoproteins

1.4.1. Definition

Lipoproteins are biochemical assemblages of proteins and lipids. Being insoluble in water, lipids transported in the circulation must be associated with proteins. Lipoprotein particles thus consist of a core of hydrophobic non-polar lipids, primarily triglycerides or cholesterol esters, surrounded by a membrane of hydrophilic polar lipids, such as phospholipids, and apolipoproteins, which are proteins that bind lipids. That the particle membrane is composed of amphipathic molecules renders lipoprotein particles water-soluble.

1.4.2. Lipoprotein classes

There are different classes of lipoprotein, distinguished by their size, composition, and apolipoproteins, and classified on their hydrated densities.[53] The six major classes are: chylomicron, very low-density lipoprotein (VLDL), intermediate-density lipoproteins (IDL), low-density lipoprotein (LDL), lipoprotein(a) [Lp(a)], and high-density lipoprotein (HDL).

Chylomicrons are the largest particles and the least dense. They are derived through the exogenous pathway, synthesised in and secreted from the small intestine in response to dietary fat intake. Chylomicrons are primarily composed of exogenous triglycerides, which are obtained from the diet and an important source of energy, containing more than twice the energy per gram as carbohydrate.[54] Triglycerides can be obtained either from dietary sources or synthesised de novo. Apolipoprotein B-48 (ApoB-48) is the major apolipoprotein of

chylomicrons. Once in the circulation, lipoprotein lipase (LPL)—present on the luminal surface of capillary endothelial cells, especially adipose and skeletal muscle tissues—hydrolyses the triglycerides into free fatty acids (FFAs). The FFAs are then absorbed by the adipocytes or myocytes to be stored or metabolised.[55] Subsequently, the now smaller, cholesterol-enriched chylomicron remnants are cleared by the liver.

Very low-density lipoproteins are derived through the endogenous pathway, produced by and released from the liver. There are a number of determinants of liver VLDL production, including availability of FFAs as a result of adipose tissue lipolysis, insulin level, and other hormonal factors.[56] They are the second largest lipoprotein, triglyceride-rich, and characterised by the presence of apolipoprotein B-100 (ApoB-100). As with chylomicrons, the VLDL triglycerides are hydrolysed by LPL and absorbed by adipocytes or myocytes.[55] As the VLDL particles become progressively smaller and cholesterol-enriched, they generate intermediate-density lipoproteins (IDLs), which are either taken up by the liver or further catabolised to LDL particles.[56] Since both chylomicrons and VLDLs have some triglycerides hydrolysed immediately as they enter the bloodstream, some consider them remnant particles once in the circulation, and their cholesterol content referred to as remnant cholesterol.[57]

Low-density lipoproteins predominantly contain cholesterol esters and are the primary transporters of cholesterol. Cholesterol is a sterol and is an important structural component of cell membranes and a substrate for the synthesis of other hormones, such as bile acids, vitamin D, and sex hormones. As with triglycerides, cholesterol can be obtained from dietary sources—chylomicron remnants—or de novo biosynthesis. Higher rates of hepatic biosynthesis of cholesterol increases VLDL production, which increases circulating LDL levels. Since they are derived from VLDL and IDL particles, LDLs carry ApoB-100 in their membranes.[58] Each chylomicron, VLDL, IDL, and LDL particle contains only one ApoB-48 (chylomicrons) or ApoB-100 (the others) molecule in its membrane. These particles are referred to as the

apolipoprotein B-containing (ApoB-containing) lipoproteins, of which approximately 90% of the circulating concentration are LDLs in normolipidaemic individuals.[59] Low-density lipoproteins are primarily cleared by the liver.

Lipoprotein(a) is formed when apolipoprotein(a) covalently binds to the ApoB-100 molecule of an LDL particle.[58] The physiological role of Lp(a) is not well understood, but it interferes with fibrinolysis and is pro-thrombotic and levels appear genetically determined. It is unclear whether clearance of Lp(a) molecules is LDL receptor-mediated or via other mechanisms.[60]

High-density lipoproteins are the smallest, most dense plasma lipoproteins and are synthesised in the liver and small intestine. They play a central role in transporting excess cholesterol from peripheral tissues to the liver, from where it can be excreted. This is known as reverse cholesterol transport (RCT).[61] HDL particles have diverse functions, including anti-inflammatory, antioxidative, and antithrombotic activities.[62] Unlike the other lipoprotein classes, apolipoprotein A-I (ApoA-I) is the major apolipoprotein of HDL particles.

1.4.3. Measurement of lipids and lipoproteins

Measuring blood lipids is incredibly common in clinical practice. Until recently a *standard lipid profile* included measures of total cholesterol (TC) concentration, total triglycerides concentration, HDL cholesterol (HDL-C) concentration, and LDL cholesterol (LDL-C) concentration.[63] The concentration of LDL-C can be calculated using the Friedewald formula [in $mmol \cdot L^{-1}$: LDL-C = TC – HDL-C – (TG/2.2)] provided triglycerides aren't elevated above 4.5 $mmol \cdot L^{-1}$.[64] The Friedewald formula calculation relies on a number of assumptions, including a constant cholesterol/triglycerides ratio (of 1/5) in VLDLs and that all triglycerides are carried by VLDL particles, both of which are not strictly true. The concentration of non-HDL-C [TC – HDL-C] can be used to estimate the total number of atherogenic lipoprotein

particles—VLDL, IDL, and LDL. Valid, accurate, and reliable measurement techniques are available for all four lipid measures.[63]

It has been standard clinical practice to draw the lipid profile after an individual has fasted to accurately measure triglycerides, and hence also to calculate LDL-C concentration if using the Friedewald formula.[65] However, evidence has accumulated over the last decade that the presumed disadvantages of non-fasting samples are negligible, and given their practicality and convenience, a number of professional societies have endorsed the use of non-fasting samples.[53]

In recent years, the causal role of ApoB-containing lipoproteins in atherosclerosis has been established and it is now desirable to measure ApoB to estimate risk and guide treatment of ASCVD.[53,66] All ApoB-containing lipoproteins contain a single ApoB molecule, hence their measurement directly estimates the number of circulating atherogenic particles. Fasting is not required, and accurate, standardised, inexpensive methods are available. Methods are also available with which to measure Lp(a), but they require standardisation before they can be considered reliable.[53] Though it can be measured, the clinical utility of ApoA-I has not been established.

Some of the previously described measures can be expressed as ratios: ApoB/ApoA-I, TC/HDL-C, non-HDL-C/HDL-C. Though each ratio may provide additional information to the standard lipid profile regarding risk, especially in individuals with particular diagnoses or pertinent family history, they cannot be used for diagnosis or treatment targets.[63]

1.5. Metabolomics

The *metabolome* is the full complement of small low-molecular-weight molecules (metabolites) in a biological sample.[67] Metabolites are produced either endogenously from chemical processes, or acquired from exogenous sources, such as diet and medication.

Endogenous metabolites include lipids, amino acids, and sugars, amongst others. *Metabolomics* is the profiling of the metabolome using either nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS). Both approaches can be run in targeted or untargeted modes.

Briefly, NMR has very high analytical reproducibility, is non-destructive to the sample which requires little preparation, and is fully automated. MS is more sensitive than NMR, but less reproducible, the data generated tend to be more complex, and it is destructive to the sample.[68] Both NMR and MS can be run in untargeted or targeted modes. Typically, the objective of the untargeted approach is discovery of new molecules, which are then identified *post hoc*. Targeted analysis is used to quantify a limited number of known metabolites and tends to be hypothesis-driven.[69] Quantitative metabolomics generates measures of metabolites in physiological and absolute units, and can therefore be analysed using standard epidemiological statistical methods.[70]

1.5.1. Lipidomics

Lipidomics refers to the analysis of the lipid complement of the metabolome—*lipidome*. In blood plasma, the lipids that comprise lipoproteins are the primary components of the lipidome.[71] Though both NMR and MS can be used to perform lipidomics, NMR is superior for analysis of lipoproteins and more commonly used.[72,73] The information generated by NMR analysis of lipoproteins is far more detailed than that obtained from the standard lipid profile. Each class of lipoproteins can be divided into a number of subclasses based on particle size, dependent on the particular NMR platform used, and produce a variety of measures for these individual lipoprotein subclasses, including particle number and lipid composition.[72] This granular data can be applied to clinical, genetic, and epidemiological studies, to improve

understanding of the mechanisms involved in lipoprotein metabolism, their association with different lifestyle and environmental exposures, and their role in disease risk.

1.6. Lipids, lipoproteins, and cardiovascular disease

Over the last century, a number of major discoveries firmly established first cholesterol, then LDL, as prime targets for the prevention of ASCVD.[74] Although the role of certain lipoproteins and their lipid content in atherosclerosis is unequivocal, the understanding of the mechanisms by which they exert their effects, and whether they are truly causal, has changed substantially in recent years.[59,75] The widespread application of novel lipoprotein measurements beyond the standard lipid profile, innovative epidemiological analysis, and disappointing clinical trial results, have led to a more nuanced appreciation of the complex role that lipoproteins play in health and disease. For example, recent evidence suggests that ApoB is likely the causal trait through which LDLs exert their well-recognised effect on ASCVD risk.[76,77] Hence, it is likely that the circulating number/concentration of these particles, rather than their lipid mass, contributes to their atherosclerotic potential.[66,78] Given that all VLDLs and IDLs, not just LDLs, contain an ApoB molecule, and all but the largest of these can cross into and be retained in the arterial intima, has stimulated interest in measuring these particles and their remnants to ascertain their role in CVD.[66,79,80] Conversely, the long-held belief that the apparent protective effect of HDL-C concentration against CVD is causal has been challenged. Despite the cholesteryl ester transfer protein (CETP) inhibitor Evacetrapid raising HDL-C levels substantially, it did not result in a lower rate of CVD events.[81] Equally, a Mendelian randomisation analysis of genetic variants known to raise HDL-C concentration showed no association with risk of myocardial infarction (MI).[82] Furthermore, epidemiological analysis of lipoprotein data obtained using NMR spectroscopy has demonstrated differing associations of lipoprotein subclasses with risk of incident CVD/CHD, contributing novel insights and

generating new hypotheses regarding the various physiological functions of lipoproteins.[83,84] Taken together, these findings illustrate both the complexity of lipoprotein metabolism and our incomplete understanding of it.

1.7. Physical activity, sedentary time, aerobic fitness and cardiometabolic health

1.7.1. Adults

There is robust evidence that higher levels of PA and aerobic fitness are strongly associated with lower risk of all-cause and CVD mortality, and ASCVD events.[85–88] There is also a convincing association between higher levels of sedentary time and all-cause and CVD mortality, though it appears at least partly dependent on PA.[37,85,89,90] Consequently, the promotion of a 'healthy lifestyle', which includes increasing PA and reducing sedentary time, is a cornerstone of CVD prevention in adults.[7,91]

1.7.2. Children

The most powerful driver of short-term (5 y to 10 y) CVD risk is age.[91] Therefore, unlike in adults, it is not feasible to measure the cardiovascular health effects of PA, time spent sedentary, and aerobic fitness against definitive clinical endpoints like CVD incidence or mortality in children. In the absence of a hereditary condition, it is rare for young people to have individual CVD risk factors, such as blood pressure or LDL-C concentration, elevated to the extent that they would warrant treatment. Furthermore, though there is some evidence that lipid levels track into adulthood and are associated with pre-clinical atherosclerosis, there is currently no direct evidence of an association between childhood risk factors and adult CVD. However, the phenomenon of CVD risk factor clustering is well-recognised in both children and adults. In adults, this clustering is defined as metabolic syndrome (MetS) when an

individual has at least three out of five risk factors (or obesity and two other risk factors, depending on which MetS diagnostic criteria are used): central obesity, hyperglycaemia, elevated blood pressure, elevated triglycerides, and decreased HDL-C concentration.[92,93] There is no consensus on a precise definition of MetS, though a number have been proposed. Nonetheless, strong associations of MetS and CVD risk, MI, type II diabetes mellitus (T2DM), and all-cause mortality have been reported in adults.[94]

1.7.3. Clinical utility of paediatric metabolic syndrome definitions

In children, there is confusion regarding not only the definition of MetS, but also its stability during the transition to adulthood, and whether as a construct it has predictive value for future health beyond that of obesity.[95] For example, though metabolic risk factor clustering during adolescence was consistent, the diagnosis of MetS was maintained to 3-y follow-up in only half of those individuals diagnosed at age 15 y.[96] Long-term stability is similarly poor. Depending on the definition of MetS used, 24-y stability of the diagnosis was between 40% to 60%.[97] The latter study found that high body mass index (BMI) alone was as good as, if not better than, dichotomous classification of MetS in childhood at predicting adult MetS, high carotid IMT, and T2DM. Furthermore, in a subsequent study that pooled a number of cohorts analysed in previous investigations of the clinical utility of paediatric MetS diagnoses, the children were stratified into age categories to determine at what age the diagnosis of MetS predicted clinical diagnosis in adulthood.[98] Paediatric MetS predicted adult risk of MetS from age 5 y, and increased adult risk of T2DM and carotid IMT from ages 8 y to 14 y. Three different definitions of paediatric MetS performed similarly, as did using individual MetS components as continuous variables. However, childhood BMI performed equally well. There was not an age at which paediatric MetS outperformed BMI at predicting adult MetS, T2DM, or carotid IMT. The clinical utility of diagnosing MetS in childhood therefore appears of

limited benefit in comparison to measuring BMI. Possible reasons for this include BMI tracking from childhood into adulthood, though the evidence for this is inconsistent;[99,100] or high BMI preceding the development of MetS, the evidence of which is also inconsistent and could be explained by BMI tracking to adulthood.[101,102] Furthermore, though PA (specifically MVPA) appears to be prospectively inversely associated with MetS and similar cardiometabolic risk scores, whether this association is independent of adiposity is unclear, and evidence of associations with individual cardiometabolic risk factors often conflicting.[103,104]

1.7.4. Consistency of associations between physical activity and lipid risk factors

Evidence from intervention studies

Since PA is strongly associated with CVD endpoints in adults, it is intuitive that PA be related to cardiometabolic risk factors in children. Yet, the evidence for this is inconsistent. Few randomised controlled trials examining PA interventions—as opposed to structured exercise training programmes—and cardiometabolic risk factors in children have been performed. In a study of first and fifth grade Swiss schoolchildren, a 9-month school-based PA intervention successfully increased daily MVPA and school day total PA in those children that received the intervention compared to the control group.[105] At follow-up, the children who received the intervention had lower composite cardiovascular risk scores, BMI, and triglycerides concentration, and higher HDL-C concentrations compared to the children in the control group. However, at follow-up 3 y subsequent to the end of the intervention, there were no between-group differences in PA levels and the only outcome where between-group differences persisted was aerobic fitness.[106] This suggests that PA interventions may need to be administered continuously to sustain their effect, though this is unlikely to be feasible. In contrast, a randomised controlled trial of a PA intervention delivered to fifth grade Norwegian

schoolchildren across one academic year reported no effect of the intervention on any individual cardiometabolic health outcomes in the main analysis, which included aerobic fitness, triglycerides concentration, and TC/HDL-C ratio, nor on clustered cardiometabolic risk score.[107] One likely reason being that there was no intervention effect on PA level compared to the control group. These inconsistent effects between studies were illustrated in a meta-analysis of school-based PA interventions.[108] The authors reported reasonable, but not unanimous, evidence across studies that PA interventions increased aerobic fitness and HDL-C levels, and decreased skinfold thickness, but a lack of consistent or conclusive evidence of an effect on BMI, body fat percentage, TC, LDL-C, or triglycerides concentration. Possible methodological explanations for these inconsistent results include heterogeneous interventions, not achieving the necessary dose of PA with the interventions, insufficient difference in PA between groups, and compensatory activity outside of school time.[109]

Evidence from observational studies

Findings from observational studies are similarly inconsistent. Consider the associations between MVPA measured using accelerometers and triglycerides concentration in children as an example. In two cross-sectional studies, one reported an association between MVPA and triglycerides concentration, whereas the other did not.[110,111] The results were similarly conflicting having mutually adjusted the regression models to include time spent sedentary, so independence of sedentary time is difficult to judge. Prospective associations are equally unclear. A study of Norwegian schoolchildren showed an inverse associated between MVPA and triglycerides concentration at follow-up having adjusted for baseline triglycerides concentration;[104] whereas another in slightly older children, followed-up over a longer period found that the inverse association was lost having adjusted for baseline concentration;[112] and a third reported that although there was an inverse association having adjusted for baseline

concentration, it was lost once time spent sedentary was included in the model.[113] There were also contrary findings for other lipid levels, for composite cardiometabolic risk scores, and differing associations with PA of different intensities.[104,114] Although methodological and analytical heterogeneity between studies is likely responsible for some of these discrepancies, it remains difficult to determine the effect of PA on these cardiometabolic risk factors due to lack of consistency of associations.

Adiposity

Whether any beneficial associations between PA and lipid risk factors are independent of adiposity is an important consideration. As previously mentioned, it could be that adiposity is the primary reason for the observed association between PA and MetS score, and that associations with other individual cardiometabolic risk markers or individual lipid levels are negligible in children. For example, although MVPA and VPA were associated with clustered cardiometabolic risk score in the prospective study of Norwegian schoolchildren described earlier, the associations were lost having removed waist circumference from the composite score and adjusted for it as a covariate.[104] Similarly, in another study MVPA was reported as being associated with MetS score independent of time spent sedentary and sleep duration, but the association was lost having adjusted for adiposity.[113]

To what extent adiposity accounts for the associations between PA and individual lipid measures is unknown. A study in children investigating whether adiposity mediated the relationship between different levels of PA and cardiometabolic risk factors reported that although there was evidence of a mediating role, the majority of the effect of PA on composite cardiometabolic risk score and individual lipid measures was explained by the direct effect of PA independent of adiposity.[115] However, given that higher levels of adiposity are strongly associated with adverse levels of a number of lipid measures in children and that a bidirectional

association has been observed between PA and adiposity, it could be that higher adiposity was responsible for lower levels of PA in the cross-sectional mediation analysis and is potentially a confounder as opposed to a mediator.[116–118] Reducing adiposity would therefore be of substantial benefit to levels of these cardiometabolic risk factors. Since PA has only a modest effect on weight loss, determining the effect of PA independent of adiposity on measures of cardiometabolic risk is important to inform the design of effective public health interventions.[119]

Sedentary time

There is some suggestion that sedentary time is associated with increased risk of CVD and unfavourable levels of certain lipid measures in adults.[90,120] A meta-analysis of interventions to reduce sedentary behaviour reported small but favourable improvements in HDL-C concentration, but not TC, LDL-C, or triglycerides concentrations.[121] Whether these associations were independent of decreased time spent sedentary and increased PA was unclear. In children, a systematic review of observational studies found that the associations between sedentary behaviour and either clustered cardiometabolic risk or individual lipid measures were inconsistent and that the associations tended to be more common with certain behaviours, such as screen time, as opposed to accelerometer-measured sedentary time.[122] Two systematic reviews and meta-analyses—one which primarily analysed cross-sectional studies, and another that examined prospective associations—reported limited to no evidence of an association between objectively measured sedentary time and cardiometabolic risk factors, especially once having accounted for MVPA.[103,123] Another meta-analysis of prospective studies, which examined different types of sedentary behaviour measured either objectively or with self-report, found no or insufficient evidence of associations of television viewing time, screen time, computer use, or objectively measured total sedentary time with any

cardiometabolic biomarkers.[124] When all of the different sedentary measures were combined, they found strong evidence of an inverse relationship with HDL-C concentration, though whether this association was independent of PA was not reported.

If daily PA as a continuous variable is associated favourably with certain cardiometabolic risk measures, then lower levels of daily PA are by definition detrimental. Given that the time in a day is finite, to increase levels of PA requires time spent in non-PA activities—sedentary time or sleep—to be reduced. Isotemporal analysis—which models the theoretical effect of replacing one activity with an equal duration of another activity—permits examination of replacing sedentary time with PA.[125] One study which modelled the associations of replacing 10 minutes of sedentary time with MVPA, found inverse associations with LDL-C concentration in children, and triglycerides concentration in adolescents, but no association with HDL-C concentration in either children or adolescents.[126] In contrast, another study reported a positive association when modelling the replacement of an hour of prolonged sedentary time (>15-min bouts) with MVPA in children.[127] Results such as these suggest that even if sedentary time is not independently associated with cardiometabolic risk factors in children, reducing time spent sedentary, if replaced with PA of sufficient intensity, could be beneficial.

Aerobic fitness

Aerobic fitness is associated with cardiometabolic risk in children. In a cross-sectional study which examined sample-derived quartiles of aerobic fitness in three European countries, strong, step-wise increases in clustered cardiometabolic risk were observed for all three countries.[128] Another study conducted in eight countries reported inverse associations between aerobic fitness and a paediatric MetS score in both cross-sectional and longitudinal analyses.[129] These associations were independent of both BMI and MVPA assessed with accelerometers.

Another cross-sectional study reported inverse associations between aerobic fitness and a MetS score independent of PA.[130] Despite associations with MetS score, associations with individual lipid measures were inconsistent in both studies.

Adiposity may partly explain the associations between aerobic fitness and individual cardiometabolic risk factors. For example, in the study that examined MetS scores including and excluding waist circumference as a component of the score, the inverse association was markedly weaker when waist circumference was excluded.[130] In another which assessed boys at three time points over two years, when expressed per kilogram of body mass, aerobic fitness was associated with favourable levels of triglycerides concentration and TC/HDL-C.[131] However, when aerobic fitness was expressed per kilogram of lean body mass, these associations were lost, which suggests that adiposity is influential to the associations between aerobic fitness and lipid measures. A systematic review of longitudinal studies found no convincing evidence of an association between aerobic fitness and lipid profile.[132] The authors stated that a number of the reviewed studies, having adjusted their analysis models for baseline measures of adiposity, reported attenuation or loss of the association between aerobic fitness and CVD risk factors. Since adiposity is a determinant of aerobic fitness and of lipid measures, it potentially confounds the associations between aerobic fitness and lipid levels.[133,134]

Though there is evidence for independent associations of aerobic fitness and PA with cardiometabolic risk factors, PA is also a determinant of aerobic fitness. However, it is potentially structured exercise training that results in improved aerobic fitness as opposed to habitual PA.[49,105,135] Furthermore, aerobic fitness is highly heritable and this heritable component is independent of the response of aerobic fitness to regular PA.[136,137] Nevertheless, there is evidence that habitual PA of a given volume is similarly beneficial at reducing incident CHD across women categorised according to genetic variants for aerobic fitness, including groups with variants associated with low baseline aerobic fitness or poor response to exercise

training.[138] In children, there is some evidence that PA moderates the association between aerobic fitness and cardiometabolic risk.[139] The association was stronger in the less fit children and independent of adiposity. Whether the benefits of engaging in sufficient PA to improve aerobic fitness are additive is not known, nor to what extent, if any, they would be independent of changes in adiposity.

1.8. Metabolomics, physical activity, sedentary time, and aerobic fitness

That there is uncertainty regarding the relationship between PA, sedentary time, aerobic fitness, and lipids and lipoproteins is evident. The potential of metabolomics to reveal novel information and improve understanding of metabolic pathways has prompted researchers to apply these techniques to existing large-scale epidemiological datasets that include measures of activity or aerobic fitness. Metabolic profiling of each exposure has shown that association magnitudes tend to differ dependent on lipoprotein subclass, which would not be revealed analysing the standard lipid panel. A notable example is of the contrasting association directions between larger and smaller HDL subclasses, which indicates that these subclasses may have distinct physiological functions that are potentially modifiable through lifestyle.[140,141] That this phenomenon was also observed with metabolic profiling of incident CVD and ischaemic stroke risk suggests it could be clinically relevant.[84] Examining metabolic phenotypes can elucidate some of the complexities of human metabolism and hopefully contribute to disentangling the independent and combined influences of lifestyle and environmental exposures on metabolic health.

2. Research motivation and aims

A number of important questions regarding the impact of PA, time spent sedentary, or aerobic fitness on CVD risk remain unanswered. These include, among others—to what extent do these behaviours or measures influence individual CVD risk factors, such as lipids and lipoproteins? are their effects independent of each other? to what degree do differences in other factors, such as adiposity, explain the associations that PA, sedentary time, or aerobic fitness have with risk factors? and do higher levels of PA or fitness, or lower levels of sedentary time in childhood confer clinical benefit in adulthood? Observing the effects of these factors at the molecular level should improve knowledge of how they impact physiology and metabolism, and therefore facilitate speculation regarding their broader influence on health and disease.

2.1. Research questions

Paper I

i. Examine the cross-sectional associations between objectively measured PA, sedentary time, and the lipoprotein profile—measured using targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.
ii. Investigate the theoretical effects on the lipoprotein profile of reallocating time spent sedentary to MVPA.
iii. Investigate the extent to which these associations are attenuated by adiposity.

Paper II

i. Examine the cross-sectional associations between aerobic fitness and the lipoprotein profile.

ii. Examine the prospective associations between baseline aerobic fitness and the lipoprotein profile at follow-up.

iii. Investigate the extent to which these associations are attenuated by adiposity.

iv. Examine the possibility that adiposity confounds the associations between aerobic fitness and the lipoprotein profile.

Paper III

i. Examine the prospective associations between baseline objectively measured PA of different intensities, sedentary time, and the lipoprotein profile at follow-up.

ii. Examine the associations between change in PA intensity, change in sedentary time, and the lipoprotein profile at follow-up.

iii. Investigate the extent to which these associations are attenuated by adiposity.

Paper IV

i. Examine whether baseline MVPA moderates the prospective associations between baseline aerobic fitness and lipoprotein subclass particle numbers at follow-up.

3. Methods

3.1. Study design

The Active Smarter Kids (ASK) study was a seven-month cluster randomised controlled trial to examine the effects of a school-based PA intervention on academic performance in fifth grade Norwegian schoolchildren (born 2004). A comprehensive description of the study design and rationale has previously been published.[30] The ASK study took place in the Sogn and Fjordane County, and commenced in 2014. Randomisation was at the school level. Sixty schools were eligible for inclusion, agreed to participate, and were randomised in a 1:1 ratio to receive either the intervention or control condition. Of these 60 schools (1202 children), three (27 children) withdrew and did not receive either the allocated intervention or control conditions. Another 46 children withdrew before testing began, hence 1129 children total were tested at baseline—93.9% of those randomised. Seven children relocated during the academic year and were unavailable for testing at the end of the intervention period.

The intervention was designed so that children within the intervention schools had the opportunity to engage in 165 $min \cdot w^{-1}$ more PA than their peers in the control schools. In addition to the obligatory 135 $min \cdot w^{-1}$ of PE and PA required by the National Curriculum—the control condition—the intervention school children received physically active lessons in three core subjects (Norwegian, mathematics, and English); PA breaks during classroom lessons; and were given PA homework. The classroom teachers delivered the intervention, having undergone training and co-developed the active lessons prior to the intervention period. The primary outcomes for academic performance in three subjects—numeracy, reading, and English—were assessed using national tests administered by the Norwegian Directorate for Education and Training (NDET). The intervention schools performed no better than the control schools in any of the academic performance outcome measures.[142] Comparing PA and

sedentary time measured using accelerometers, no differences between conditions were observed and baseline PA levels were high, which likely contributed to the intervention's lack of effect.

Given the absence of between-group differences in PA and sedentary time, the entire sample was pooled as one cohort for subsequent analyses. Flow of the ASK study cohort with regard to the research objectives of the present thesis is illustrated in Figure 1. A full description of participant flow is provided in the main outcomes paper.[142] The ASK study is registered at https://clinicaltrials.gov, #NCT02132494.

3.2. Ethics

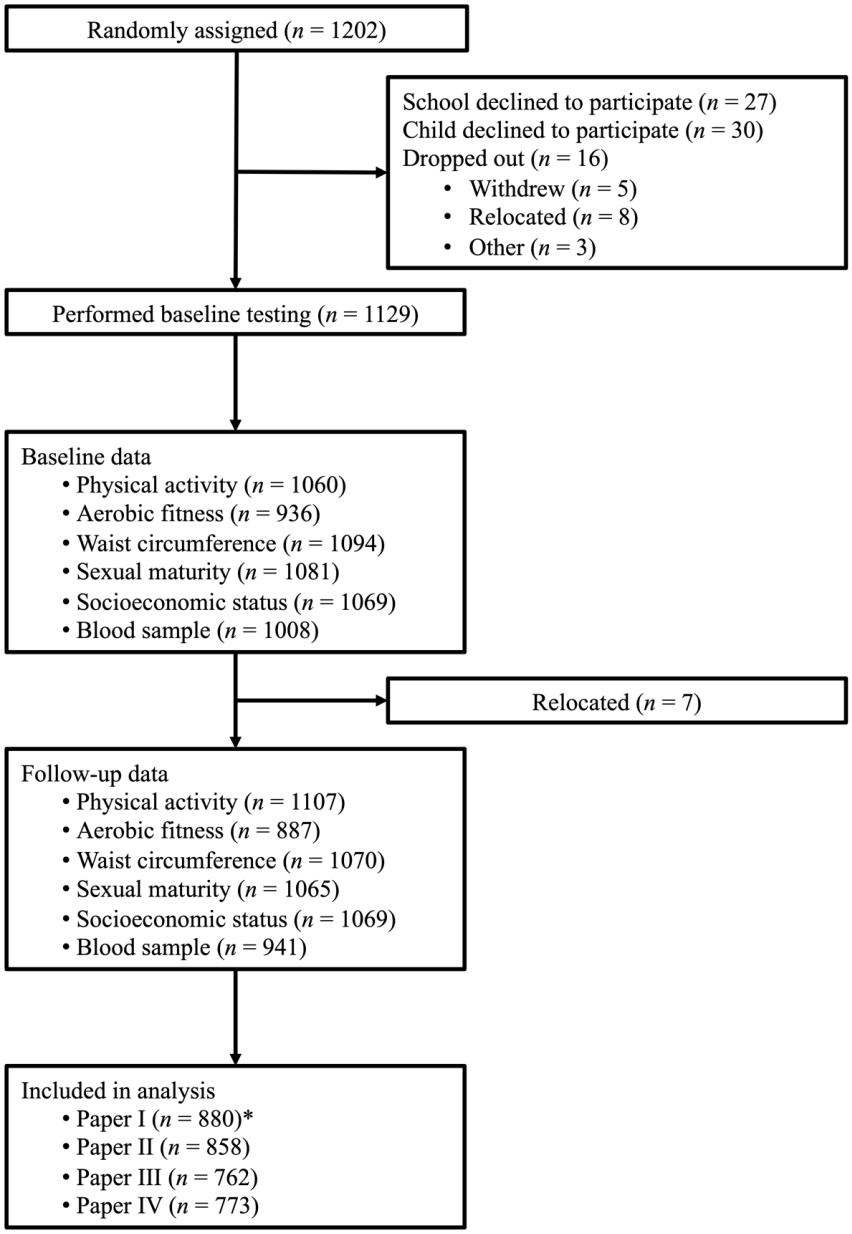
The Regional Committee for Medical Research Ethics approved the ASK study protocol (Appendix 1). Printed study information sheets were distributed, and written consent was obtained from each child's parent(s) or legal guardian(s) and from school authorities prior to testing (Appendix 2). All study procedures and methods used abide by the World Medical Association's Declaration of Helsinki.[143]

3.3. Measures

3.3.1. Physical activity and sedentary time

The children wore triaxial accelerometers (ActiGraph GT3X+, ActiGraph 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.[144] The accelerometer data were processed using commercially available KineSoft software (version 3.3.80, KineSoft, Loughborough, United Kingdom) and 10-s epochs.

Figure 1. Flow of participants analysed in Papers I–IV



*In addition to the 1129 children that underwent baseline testing, data for an additional 59 children (1 school) were available for analysis in Paper I. They were not included in any of the subsequent papers.

Physical activity intensity and sedentary time were classified using the Evenson cut points of count data: sedentary time (≤100 counts·min^{-1}), low-intensity physical activity (LPA; >100 and <2296 counts·min^{-1}), moderate-intensity physical activity (MPA; ≥2296 and <4012 counts·min^{-1}), and vigorous-intensity physical activity (VPA; ≥4012 counts·min^{-1}).[145,146] Moderate- to vigorous-intensity PA included all activity above the MPA cut point (≥2296 counts·min^{-1}).

3.3.2. Aerobic fitness

Aerobic fitness was assessed using the Andersen intermittent shuttle run test.[48] The objective of the Andersen test is to cover the greatest distance possible in 10 minutes. Following an explanation of the test and five-minute warm-up, the children ran back and forth between two parallel lines set 20 m apart, alternately running for 15 s then pausing for 15 s. Each time they reached either end line, the children had to touch beyond it with one finger before they could run back in the opposite direction. Study research assistants supervised testing and recorded the total distance (m) covered for each child. Distance run was used as the main exposure as opposed to applying a prediction equation to convert distance run to an estimate of $\dot{V}O_{2peak}$.

3.3.3. Lipoprotein measures

Blood samples

The children fasted overnight and a trained nurse or phlebotomist drew the samples between 0800 and 1000. Serum was drawn from an antecubital vein and obtained according to a standard protocol: blood plasma was collected in 5 ml VACUETTE Serum Gel with Activator blood collection tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria); the tubes were carefully inverted five times and placed vertically for coagulation; after 30 minutes, the samples were centrifuged at 2000 G for ten minutes and the serum visually inspected for

residue and centrifugation repeated if present; the tubes were kept in a refrigerator at 4°C before pipetting 0.5 ml into cryo tubes; the cryo tubes were then stored in a freezer at –20°C for up to 2 days before finally being stored at –80°C until analysis.

^{1}H NMR protocol

NMR 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). The frozen serum samples were thawed at room temperature for approximately 1 h. Aliquots of 120 µl were carefully mixed with equal amounts of phosphate buffer in Eppendorf tubes, and transferred to 3 mm Bruker NMR SampleJet tubes by syringe. A fill height of 4 cm was used amounting to approximately 180 µl. Serum spectra were recorded at 310 K, using a one-dimensional NOESY (noesygppr1d) pulse sequence. A total of 32 scans were acquired, using 96k data points and 30 ppm spectral width. The spectra were processed with 0.3 Hz line broadening, automatically phase-corrected and aligned to the lactate signal at 1.32 ppm. Spectra were normalised to an ERETIC signal, functioning as an external reference.

Spectral regions quantitatively associated to lipoprotein concentrations were selected as explanatory variables to partial least squares (PLS) modelling and performed for 106 randomly selected serum samples analysed by both NMR and high-performance liquid chromatography (HPLC).[147] A Monte Carlo resampling approach was used to calculate individual PLS models with optimal prediction ability for the HPLC data.[148] Lipoprotein particle numbers for all samples were subsequently predicted from these models, and the 20 lipoprotein subclasses were reduced to 15.[149] 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.[150] Lipoprotein measures available for subsequent analysis comprised: total serum cholesterol concentration; total triglycerides concentration; non-HDL-C concentration; particle number,

cholesterol concentration and triglycerides concentration of 15 lipoprotein subclasses; and average particle diameter of VLDL, LDL, and HDL subclasses. 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. 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.[65]

3.3.4. Anthropometrics

The children wore light clothing for the assessment, shoes removed. The height of each child was measured to the nearest 0.1 cm using a portable stadiometer (Seca 217, SECA GmbH, Hamburg, Germany), facing forward; weight to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany); and waist circumference to the nearest 0.1 cm using a measuring tape (Seca 201, SECA GmbH, Hamburg, Germany). For waist circumference, two measurements were made between the lowest palpable rib and iliac crest, the child having gently exhaled. If there was a difference of more than 1.0 cm between the two measurements, a third was taken. The mean of the two measurements with the least difference was recorded for analysis. Body mass index was calculated as the weight divided by the square of height ($kg \cdot m^{-2}$). In Papers II, III, and IV, the International Obesity Task Force's (IOTF) sex-specific BMI cut-off values were used to calculate the proportion of overweight and/or obese children, rounding each child's age down to the nearest half-year.[151]

3.3.5. Sexual maturity

Each child assessed their own sexual maturity against a standard set of images and text descriptions that correspond to the Tanner staging method.[152] The assessments took place in a private room and the children were accompanied by a researcher of the same sex to ensure their

comfort. Low frequencies of children in Tanner categories 3, 4, and 5 (n = 66, 5, 2, respectively of 1081 children with valid baseline data) were recorded and therefore combined into one category (≥3). In Papers II, III, and IV, sexual maturity was the Tanner score for pubic hair development for both girls and boys. In Paper I, girls were assigned a single score for whichever was the higher of pubic hair development or breast development.

3.3.6. Socioeconomic status

Socioeconomic status (SES) was considered as the highest level of attainment of a child's mother, father, or guardian, whichever was higher. Parent(s) or guardian(s) individually completed a custom self-report study 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, 137, for categories 1–4, respectively of 1069 children with valid baseline data), so were combined into one category—*Upper secondary school*—for analysis.

3.4. Statistics

All analyses were conducted using R (R Foundational for Statistical Computing, Vienna, Austria [version 3.4.3 in Paper I; version 3.6.3 in Papers II, III, and IV]).[153]

Paper I

Cross-sectional associations between the mean daily time spent in different intensities of activity for three activity intensities (LPA, MVPA, and sedentary time) and the 30 lipoprotein variables measured at baseline were examined using separate linear models (median regression models for skewed lipoprotein variables). Each model was adjusted for sex and parents' education, and baseline value of mean daily accelerometer wear time, age, and sexual maturity.

To investigate potential confounding by adiposity, baseline waist circumference was included in the model and the analysis repeated. The MVPA model was subsequently adjusted for sedentary time and vice versa to examine whether the associations of one behaviour were independent of the other.

All lipoprotein variables were converted to *z*-scores (mean = 0.0; standard deviation [SD] = 1.0) and all activity variables were standardised to 30 minutes. Hence, regression coefficients represent the SD unit change in lipoprotein measure for a 30 $min \cdot d^{-1}$ increase in activity variable.

Isotemporal substitution models were employed to examine the effect of replacing time spent sedentary with an equal amount of MVPA. An isotemporal substitution analysis simultaneously models both the activity being performed and the activity being replaced in an equal time-exchange manner, whilst holding other activity types constant.[125] For example, excluding sedentary time from a regression model that keeps MVPA, LPA and monitor wear time constant, the coefficient obtained for MVPA demonstrates the theoretical effect of replacing sedentary time with a specified amount of MVPA. Hence, regression coefficients represent the SD unit change in lipoprotein measure for a 30 $min \cdot d^{-1}$ substitution of MVPA to replace 30 minutes of sedentary time. These models included the same covariates as the cross-sectional models.

A false discovery rate (FDR) correction was applied using the Benjamini-Hochberg procedure.[154] The resulting *q* values were interpreted at an alpha threshold of 0.05 ($q < 0.05$). For each lipoprotein measure, the coefficients were reported in both absolute units and *z*-score units for a 30-minute increment in activity. The 95% confidence intervals (CIs) were given in *z*-score units.

Paper II

Cross-sectional associations between aerobic fitness and 57 lipoprotein variables measured at baseline were examined using separate linear regression models. Each model was adjusted for sex and parents' education, and the baseline value of age and sexual maturity.

Prospective associations were examined by regressing 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 also included. The analyses were repeated including baseline waist circumference in the model to investigate potential confounding by adiposity. Cross-sectional and prospective associations between waist circumference and aerobic fitness were also examined, adjusting for the same covariates as in the aerobic fitness models, and additionally for aerobic fitness at baseline in the prospective model.

To compare the magnitudes of main effects of aerobic fitness and waist circumference, cross-sectional and prospective associations between waist circumference and the lipoprotein profile were examined, adjusting for the same covariates as in the aerobic fitness models.

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.[155,156] 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% CIs were given in standardised units.

Paper III

Prospective associations between the mean daily time spent in different intensities of activity for four activity intensities (LPA, MPA, VPA, and sedentary time) measured at baseline and the 57 lipoprotein variables measured at follow-up using separate linear models were examined. Each model was adjusted for sex and parents' education, and the baseline value of the respective lipoprotein measure, mean daily accelerometer wear time, age, and sexual maturity.

To examine the associations with change in mean daily time spent in different intensities of activity over the follow-up period, change scores were used (follow-up minus baseline). Accelerometer wear time was also modelled as a change score. Baseline values were used for the other covariates.

The analyses were repeated including baseline waist circumference in the model to investigate potential confounding by adiposity.

All PA and lipoprotein variables were converted to *z*-scores (mean = 0.0; SD = 1.0). In the change models, changes in mean daily time spent in different intensities of activity were modelled as the *z*-score (follow-up minus baseline). 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. The multiple testing correction was the same as in Paper II. Hence, the Bonferroni-corrected alpha threshold for assessing associations was 0.05/5 = 0.01 (p <0.01). For each lipoprotein measure, the coefficients were reported in both absolute units and standardised units. The 95% CIs were given in standardised units.

Paper IV

Prospective associations between aerobic fitness measured at baseline and the 15 lipoprotein subclass particle numbers variables measured at follow-up were examined using separate linear models. Each model was adjusted for sex, and parents' education, and the baseline values of mean daily MVPA, the respective lipoprotein measure, age, and sexual maturity.

All lipoprotein variables, aerobic fitness, and MVPA were converted to *z*-scores (mean = 0.0; SD = 1.0). 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 examine whether MVPA moderated the effect of aerobic fitness on the lipoprotein measures, an interaction term (aerobic fitness x MVPA) was included in each model and a *simple slopes* analysed, examining each regression model fixed at three sample-estimated values of MVPA: mean, and mean ± 1 SD.[157] The calculations were averaged over the levels of the categorical variables: parents' education, sex, and sexual maturity. Mean values were used for each continuous variable: age and baseline lipoprotein variable. Simple slopes represent the change in the *z*-score lipoprotein variable per SD unit increase in aerobic fitness whilst holding MVPA constant at each of the three levels specified. The interactions were also examined visually, plotting the simple slopes at SD unit increments across the range of distances run in the Andersen test. The hypothesis was that were MVPA to have a moderating effect on the association between aerobic fitness and lipoprotein subclass particle numbers it would be more marked at lower levels of fitness. Therefore, pairwise comparisons of predicted values for each lipoprotein measure between ±1 SD of MVPA were performed, at –1 SD of the Andersen test. The same multiple testing correction was applied as in Papers II and III. Hence, the Bonferroni-corrected alpha threshold for assessing associations was 0.05/5 = 0.01 (*p*

<0.01). For each lipoprotein measure, the coefficients were reported in both absolute units and standardised units. The 95% CIs were given in standardised units.

4. Results

The figures and tables referred to in the Results section are presented either inline or in the Supplementary material section. Additional data tables and figures are available in Papers I–IV.

4.1. Characteristics of the study sample

The characteristics of the ASK study cohort split at both measurement occasions are presented in Table 1. The analytical sample varied dependent on the particular research objectives of each of the four included papers. Readers are referred to the individual papers for further information.

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

	Baseline		Follow-up	
Characteristic	*n* (%)	Mean (SD)	*n* (%)	Mean (SD)
Age (years)	1077	10.0 (0.3)	1117	10.9 (0.3)
Sex				
Girls	541 (47.9)			
Boys	588 (52.1)			
Anthropometrics				
Height (m)	1096	142.7 (6.8)	1069	146.7 (7.1)
Weight (kg)	1095	37.0 (8.1)	1072	39.5 (8.7)
BMI ($kg \cdot m^{-2}$)	1095	18.1 (3.0)	1069	18.2 (3.0)
≥25	235 (21.5)		223 (20.9)	
≥30	45 (4.1)		43 (4.0)	
Waist circumference (cm)	1094	62.0 (7.5)	1070	63.2 (7.8)
Parents' education	1069			
Upper secondary school	349 (32.6)			
<4 years college/university	320 (29.9)			
≥4 years college/university	400 (37.4)			
Tanner stage	1081		1065	
Stage 1	581 (53.7)		392 (36.8)	
Stage 2	427 (39.5)		532 (50.0)	
Stage ≥3	73 (6.8)		141 (13.2)	
Physical activity	1060		1107	
VPA ($min \cdot d^{-1}$)		30.8 (16.0)		26.5 (13.4)
MPA ($min \cdot d^{-1}$)		44.2 (13.2)		40.2 (12.4)
LPA ($min \cdot d^{-1}$)		232.7 (38.6)		220.0 (36.4)
SED ($min \cdot d^{-1}$)		466.7 (59.7)		492.3 (57.2)
≥60 MVPA ($min \cdot d^{-1}$)	752 (66.6)		663 (58.7)	
Aerobic fitness	936		887	
Andersen test (m)		896.8 (59.7)		937.0 (57.2)
Lipid profile	1008		941	
TC ($mmol \cdot L^{-1}$)		4.5 (0.7)		4.5 (0.6)
LDL-C ($mmol \cdot L^{-1}$)		2.5 (0.6)		2.6 (0.6)
HDL-C ($mmol \cdot L^{-1}$)		1.6 (0.3)		1.6 (0.3)
TG ($mmol \cdot L^{-1}$)[a]		0.7 [0.5, 0.9]		0.7 [0.5, 0.9]

[a]Median [IQR].

Abbreviations: BMI = body mass index; HDL-C = high-density lipoprotein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; LPA = light-intensity physical activity; MPA = moderate-intensity physical activity; MVPA = moderate- to vigorous-intensity physical activity; SD = standard deviation; SED = sedentary time; TC = total cholesterol; TG = triglycerides; VPA = vigorous-intensity physical activity.

4.2. Paper I

4.2.1. Cross-sectional associations between physical activity and the lipoprotein profile

A 30-minute increment in MVPA was associated with a number of the 30 lipoprotein measures (Figure 2 and Supplementary table 1). The associations were inverse with all but one measures of the VLDL subclasses (e.g., –1.27 x 10^{-1} nmol·L^{-1} or –0.11 SD; 95% CI = –0.17, –0.04; *q* <0.01 for VLDL L1 subclass particle number). Apart from a marked positive association with the average diameter of LDL particles, those with the other LDL measures were typically modest. In addition to a positive association with average diameter of HDL particles, there were positive associations with the three subclasses of the largest particles, and inverse but weaker with the HDL S and HDL VS subclasses. Regarding the more traditional lipid measures, there was an inverse association with total triglycerides concentration, a positive association with HDL-C concentration, and negligible associations with LDL-C and TC concentration.

Having additionally included waist circumference as a covariate in the models, the associations were attenuated for a number of individual lipoprotein measures. The degree of attenuation tended to be less pronounced for the VLDL particle measures (e.g., –1.22 x 10^{-1} nmol·L^{-1} or –0.10 SD; 95% CI = –0.14, –0.06; *q* <0.001 for VLDL L1 subclass particle number).

The associations between a 1 SD increment in LPA were typically weak (Figure 3 and Supplementary table 2).

4.2.2. Cross-sectional associations between sedentary time and the lipoprotein profile

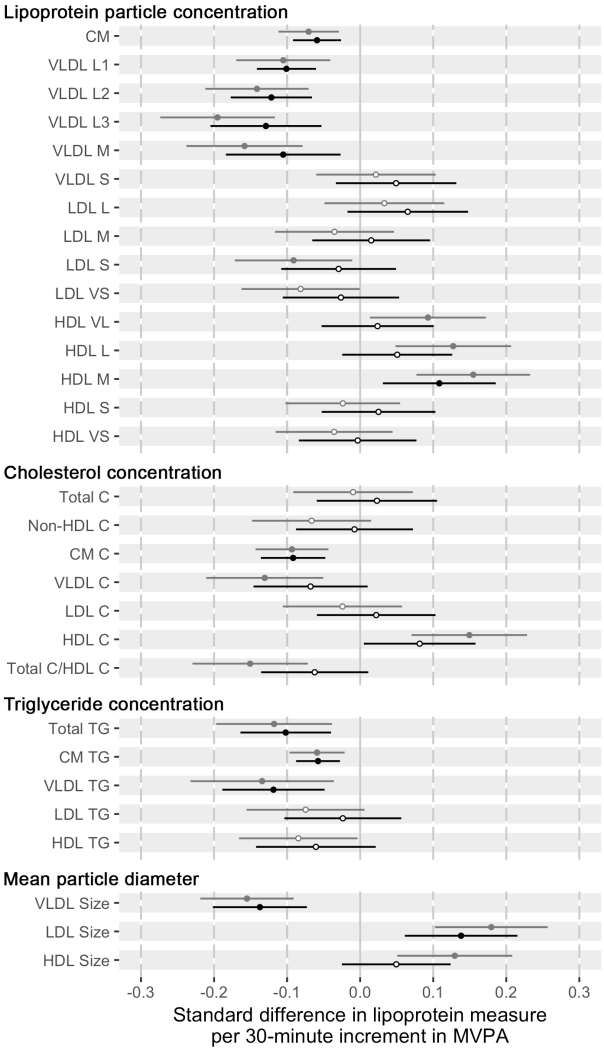
The associations between a 30-minute increment in time spent sedentary were modest to negligible (Figure 4 and Supplementary table 3). Those associations that were more

pronounced tended to be with the measures of VLDL particles (e.g., 4.20 x 10^{-2} nmol·L^{-1} or 0.03 SD; 95% CI = 0.01, 0.06; q <0.05 for VLDL L1 subclass particle number). For these measures, the directions of associations were the opposite of those in the MVPA model. Typically, the degree of attenuation was negligible having included waist circumference as a covariate in the models.

4.2.3. Isotemporal substitution of moderate- to vigorous-intensity physical activity for sedentary time

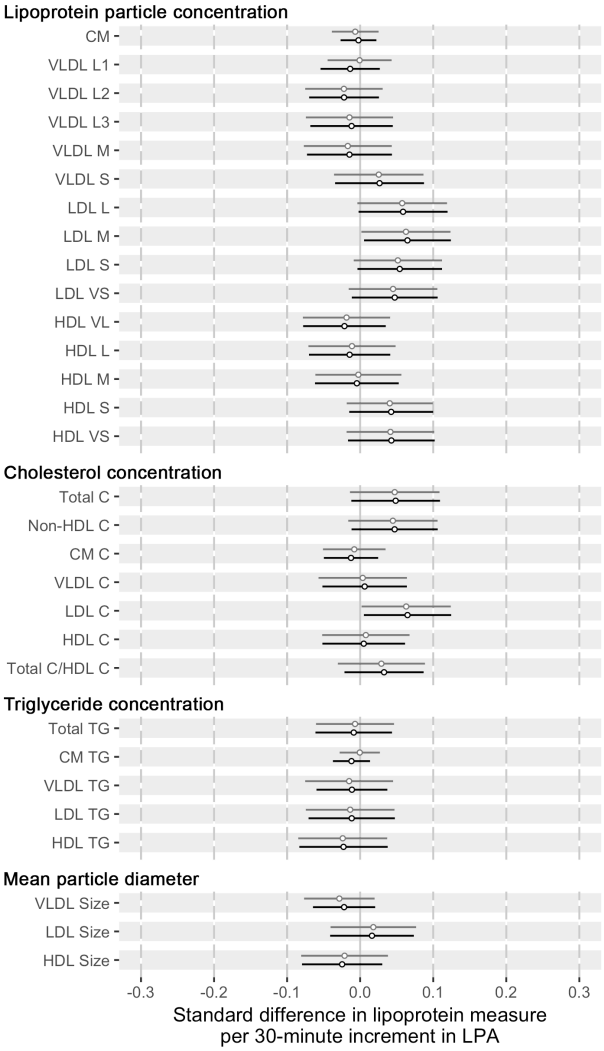
The pattern of associations for a reallocation of 30 minutes time spent sedentary to an additional 30 minutes of daily MVPA was very similar to that of the single activity MVPA model (Figure 5 and Supplementary table 4).

Figure 2. Cross-sectional associations of time spent in MVPA with 30 serum lipoprotein measures



The associations were adjusted for monitor wear time, parents' education, sex, and sexual maturity (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in MVPA. Filled circles are FDR-corrected *p* value <0.05. Error bars are 95% CIs. Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

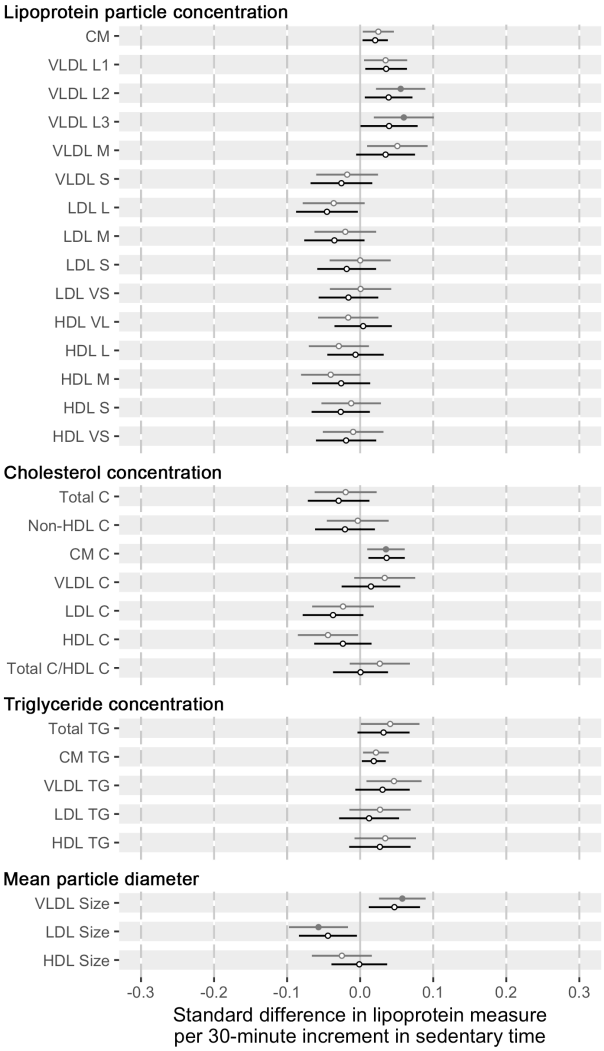
Figure 3. Cross-sectional associations of time spent in LPA with 30 serum lipoprotein measures



The associations were adjusted for adiposity, monitor wear time, parents' education, sex, and sexual maturity (grey). Analyses were additionally adjusted for sedentary time (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in MVPA. Filled circles are FDR-corrected *p* value <0.05. Error bars are 95% CIs.

Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

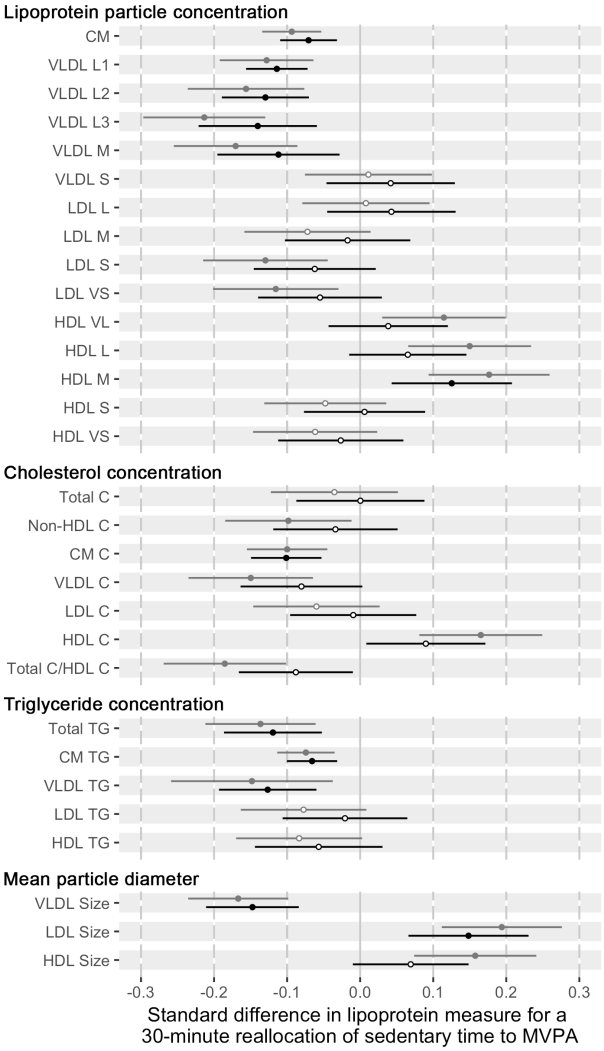
Figure 4. Cross-sectional associations of sedentary time with 30 serum lipoprotein measures



The associations were adjusted for monitor wear time, parents' education, sex, and sexual maturity (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in sedentary time. Filled circles are FDR-corrected p value <0.05. Error bars are 95% CIs.

Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 5. Cross-sectional associations of 30 serum lipoprotein measures with an isotemporal substitution of 30 minutes time spent in MVPA for 30 minutes of sedentary time



The associations were adjusted for monitor wear time, parents' education, sex, and sexual maturity (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure for a 30-minute reallocation of activity. Filled circles are FDR-corrected *p* value <0.05. Error bars are 95% CIs. Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

4.3. Paper II

4.3.1. Cross-sectional associations between aerobic fitness and the lipoprotein profile

Aerobic fitness was associated with all but one of the 57 lipoprotein measures analysed (Figure 6 and Supplementary table 5). For a 1 SD increment in distance run for the Andersen test (103 m), there were inverse associations with every measure of ApoB-containing lipoproteins, which included subclass particle numbers, and cholesterol and triglycerides concentrations (e.g., –2.61 x 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 the HDL measures, the associations tended to differ dependent on subclass. Those with particle number, cholesterol concentration, and triglycerides concentration 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 TC, non-HDL-C, and LDL-C cholesterol concentrations, and total triglycerides concentration, and positively associated with HDL-C 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 (Figure 7 and Supplementary table 6). 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.

4.3.2. Prospective associations between aerobic fitness and the lipoprotein profile

The directions of associations remained the same as those in the cross-sectional analysis (Figure 8 and Supplementary table 7). Effect sizes for all but one were attenuated. The degree of attenuation varied considerably between individual measures. Comparing the prospective and cross-sectional analyses, the associations between a 1 SD increment in distance run (102 m) with the VLDL subclasses were typically more consistent than with the LDL and HDL measures (e.g., $–1.73 \times 10^{–1}$ 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 with the four measures of cholesterol concentration.

The pattern of associations remained broadly similar having included waist circumference as an additional covariate in the prospective models (Figure 9 and Supplementary table 8). The effect sizes were generally more modest than in the other models but tended to be larger for the larger ApoB-containing particles (e.g., $–7.74 \times 10^{–2}$ 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.

4.3.3. Associations between waist circumference and the lipoprotein profile

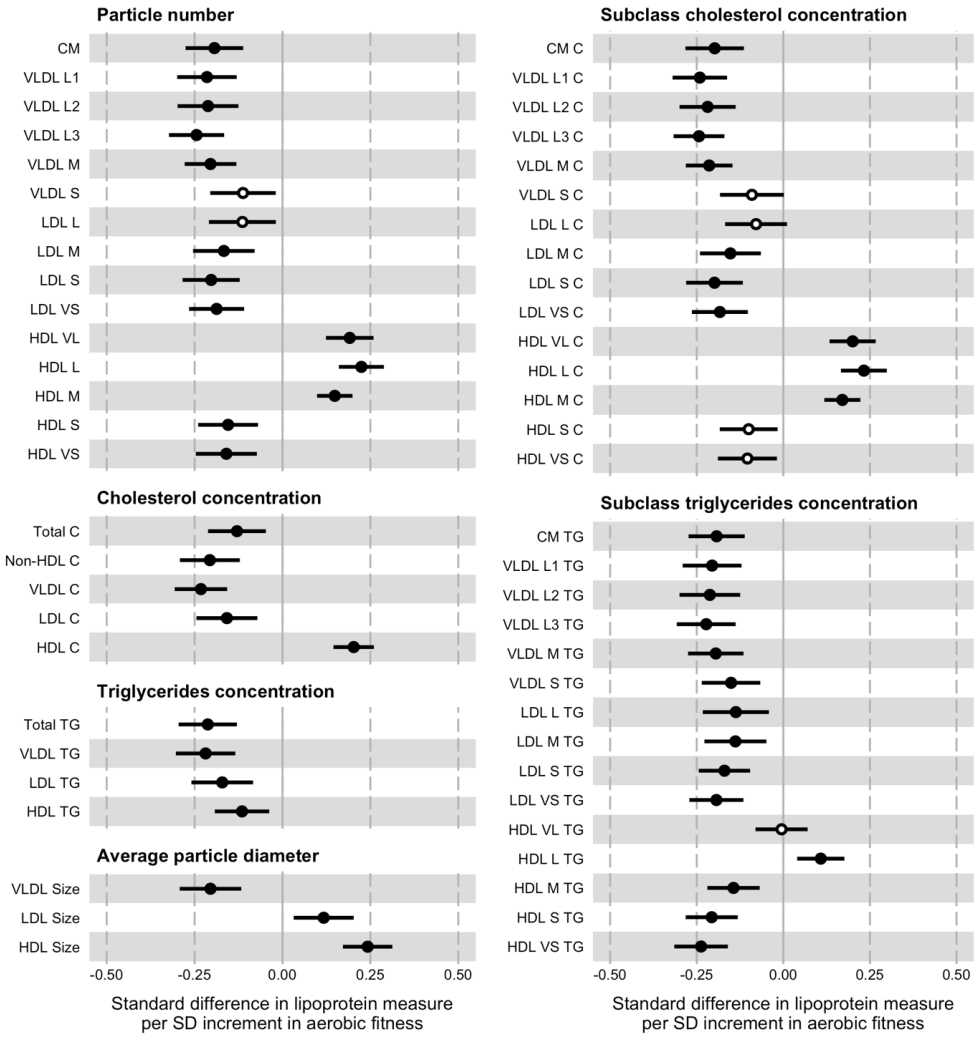
For all but one of the 57 measures, the directions of association with a 1 SD increment in waist circumference (7.5 cm) were the opposite of those in the cross-sectional analysis of aerobic fitness not adjusted for waist circumference (Figure 10 and Supplementary table 9). The pattern of associations was broadly similar. The effect sizes were larger in the waist circumference model for all but two of the lipoprotein measures (e.g., 3.56 x 10^{-1} nmol·L^{-1} or 0.29 SD; 95% CI = 0.18, 0.36; p <0.001 for VLDL L1 subclass particle number).

Similarly, when comparing the prospective models of waist circumference with aerobic fitness not adjusted for waist circumference, the directions of association were the opposite for all but one lipoprotein measure, and effect sizes larger in the waist circumference model for all but three (Figure 11 and Supplementary table 10).

4.3.4. Associations between waist circumference and aerobic fitness

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) (Supplementary tables 11 and 12).

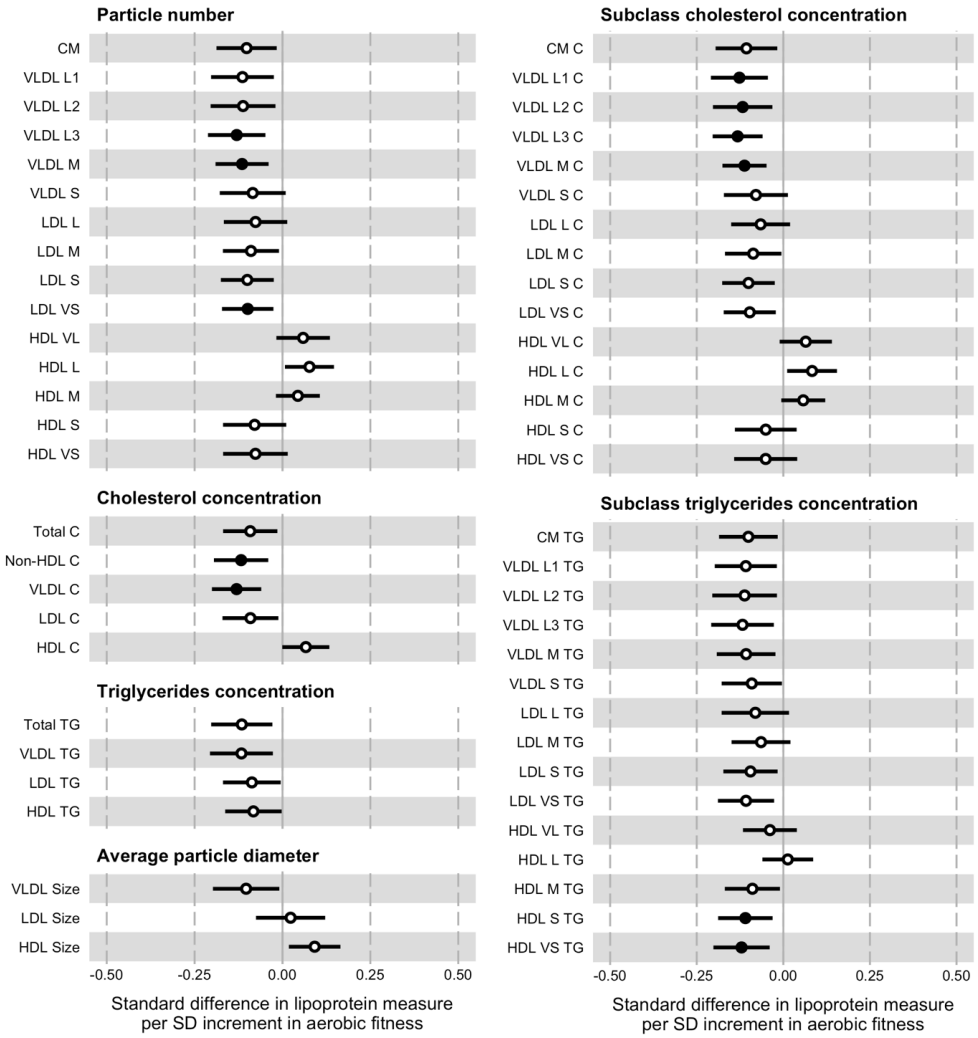
Figure 6. Cross-sectional associations of aerobic fitness with 57 lipoprotein measures



The associations were adjusted for age, parents' 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.

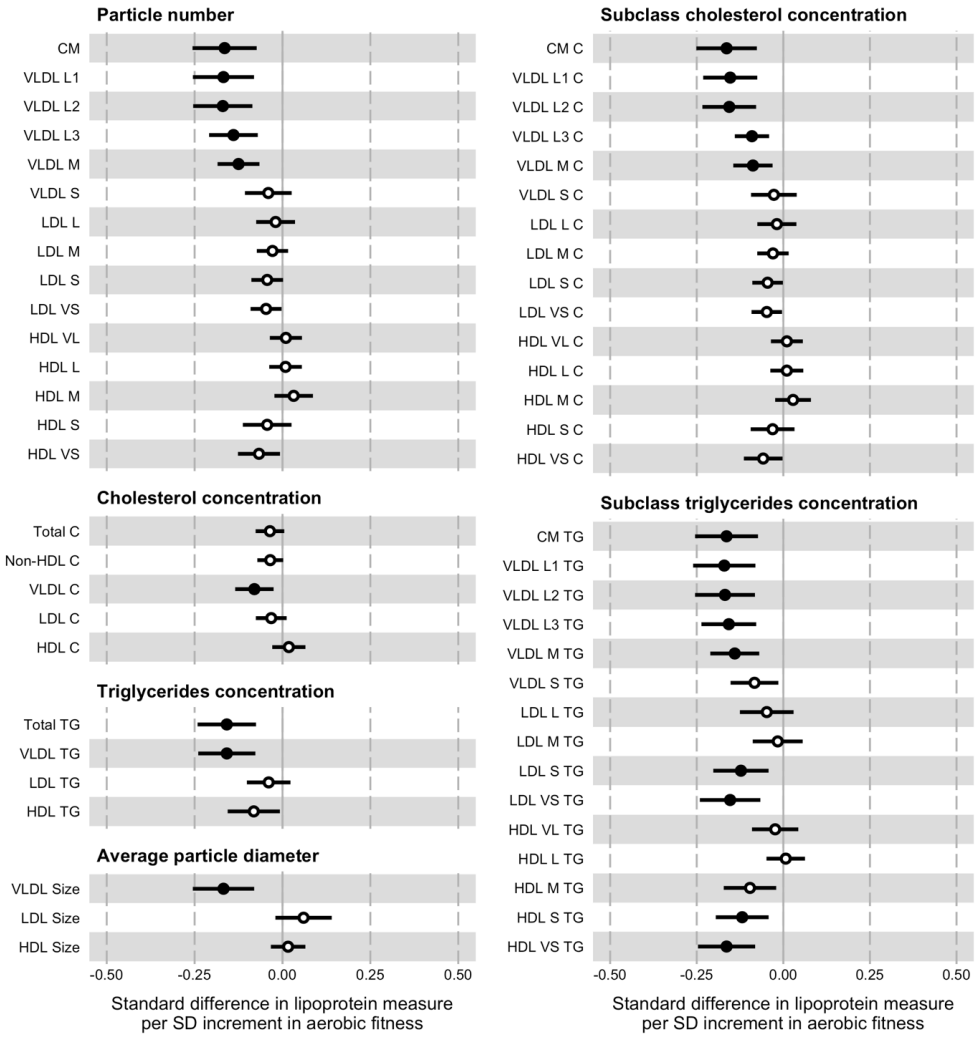
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 7. Cross-sectional associations of aerobic fitness with 57 lipoprotein measures, adjusted for waist circumference



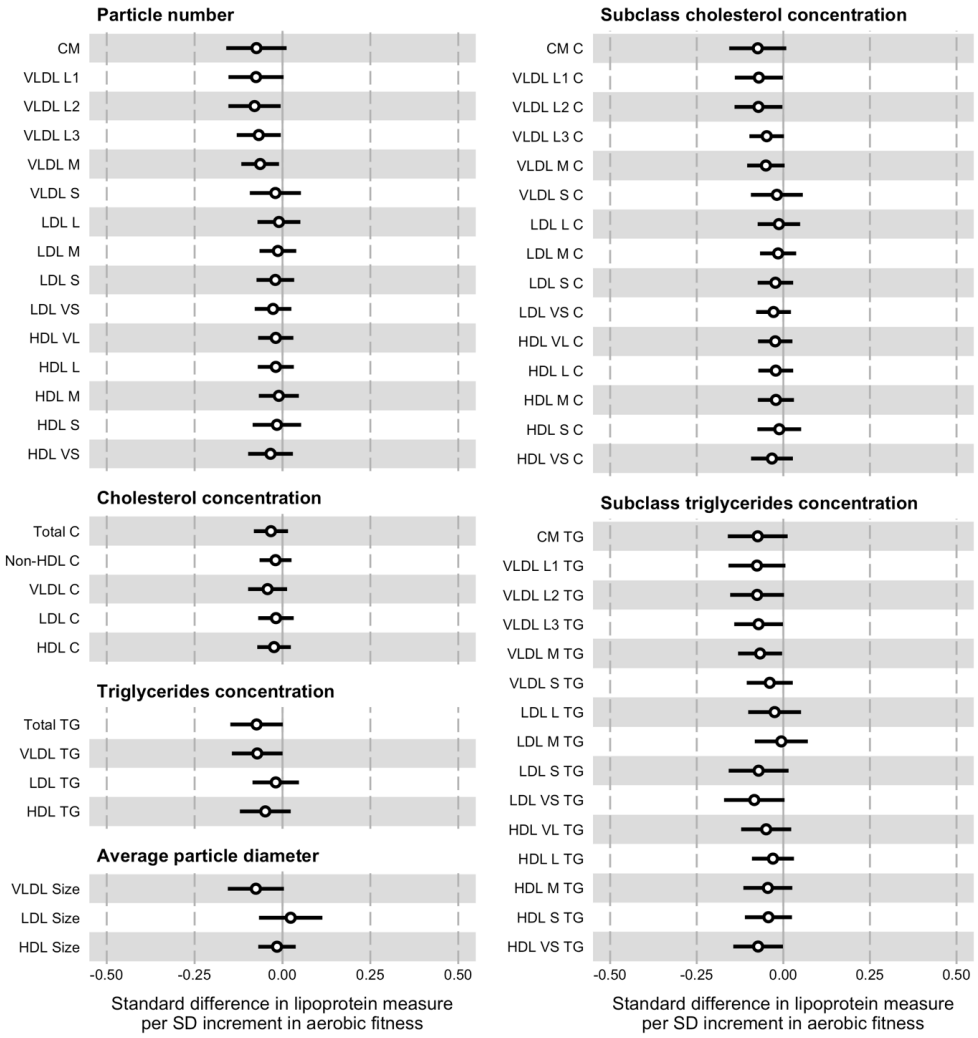
The associations were adjusted for age, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in aerobic fitness. Filled circles are p <0.01. Error bars are 95% CIs.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 8. Prospective associations of aerobic fitness with 57 lipoprotein measures



The associations were adjusted for age, parents' education, sex, sexual maturity, and baseline lipoprotein measure. 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. Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

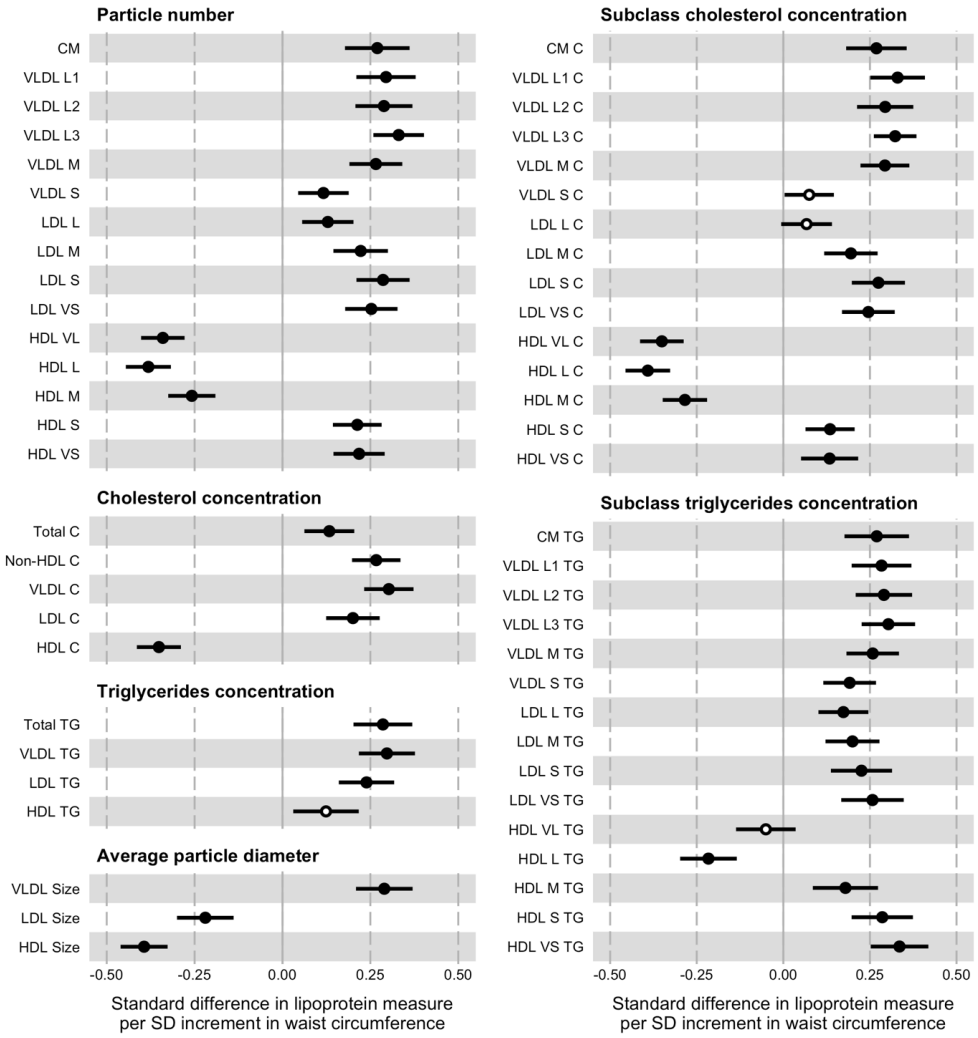
Figure 9. Prospective associations of aerobic fitness with 57 lipoprotein measures, adjusted for waist circumference



The associations were adjusted for age, parents' education, sex, sexual maturity, baseline lipoprotein measure, 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.

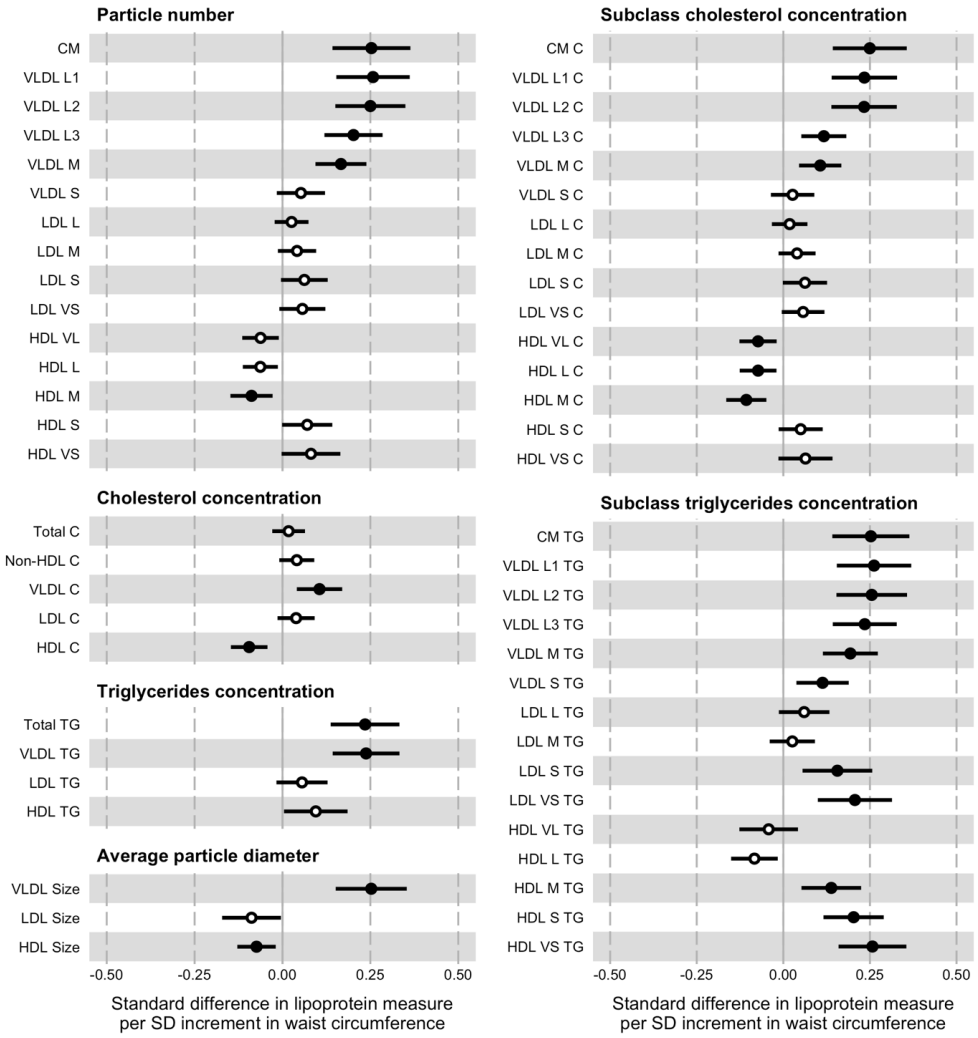
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 10. Cross-sectional associations of waist circumference with 57 lipoprotein measures



The associations were adjusted for age, parents' 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 waist circumference. Filled circles are p <0.01. Error bars are 95% CIs.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 11. Prospective associations of waist circumference with 57 lipoprotein measures



The associations were adjusted for age, parents' education, sex, sexual maturity, and baseline lipoprotein measure. 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 waist circumference. Filled circles are p <0.01. Error bars are 95% CIs.

Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

4.4. Paper III

4.4.1. Associations between vigorous-intensity physical activity and the lipoprotein profile

In the prospective analysis, there were pronounced inverse associations between a 1 SD increment in VPA (16.2 min·d^{-1}) and all but one measure of the VLDL particles (Figure 12 and Supplementary table 13). For the individual measures of particle number, cholesterol concentration, and triglycerides concentration the effect sizes decreased from the largest to smallest of these particles (e.g., –1.32 x 10^{-1} nmol·L^{-1} or –0.13 SD; 95% CI = –0.19, –0.06; p <0.001 for VLDL L1 particle number). The associations between VPA and LDL measures were also inverse, though effect sizes smaller than for the measures of larger ApoB-containing particles. The triglycerides concentrations of the two subclasses of the smallest LDL particles were an exception. For the HDL measures, the directions of associations tended to differ dependent on subclass. The effect sizes were mostly modest, though larger for the triglycerides concentrations of the two subclasses of the smallest HDL particles. The association with the average diameter of VLDL particles was inverse and effect size larger than those of the positive associations with the average diameter of LDL and HDL particles. Regarding the more traditional lipid measures, higher VPA was inversely associated with TC, non-HDL-C, and LDL-C concentrations, and positively associated with HDL-C concentration. The effect sizes were modest. In contrast, the effect size was larger for the inverse association with total triglycerides concentration. The pattern of associations remained broadly similar having included waist circumference as an additional covariate in the models. The degree of attenuation for individual measures ranged from small to moderate and tended to be greater for the subclasses of larger particles (–8.60 x 10^{-2} nmol·L^{-1} or –0.08 SD; 95% CI = –0.14, –0.03; p <0.01 for VLDL L1 particle number).

The associations with a 1 SD change in VPA (14.9 min·d^{-1}) between baseline and follow-up measurement occasions were weak (Figure 13 and Supplementary table 14). Adjustment for waist circumference had a negligible effect on these associations.

4.4.2. Associations between moderate-intensity physical activity and the lipoprotein profile

In the prospective analysis, the pattern of associations between a 1 SD increment in MPA (12.8 min·d^{-1}) and the lipoprotein measures was broadly similar to that of a 1 SD increment in VPA (Figure 14 and Supplementary table 15). The effect sizes were smaller for all but four of the 57 individual measures (e.g., –1.14 x 10^{-1} nmol·L^{-1} or –0.11 SD; 95% CI = –0.19, –0.03; *p* <0.01 for VLDL L1 particle number). Many of the coefficients were almost zero. Including waist circumference as an additional covariate in the models had a negligible effect.

Generally, the pattern of associations with a 1 SD change in MPA (12.1 min·d^{-1}) was similar to those in the VPA change model, though the effect sizes for many individual measures were larger (Figure 15 and Supplementary table 16). Adjustment for waist circumference had a negligible effect on these associations.

4.4.3. Associations between light-intensity physical activity and the lipoprotein profile

In contrast to the VPA and MPA analyses, the associations between a 1 SD increment in LPA (36.7 min·d^{-1}) and the VLDL measures tended to be more modest (Figure 16 and Supplementary table 17). The effect sizes with certain individual subclasses were small, and those with the VLDL L3 and VLDL M subclasses almost zero (e.g., –4.53 x 10^{-2} nmol·L^{-1} or –0.04 SD; 95% CI = –0.12, 0.03; *p* 0.24 for VLDL L1 particle number). In contrast to the VPA and MPA analyses, the directions of associations with the LDL subclass measures tended to be

positive, though the distinct inverse associations with the triglycerides concentrations of the LDL S and LDL VS subclasses were replicated. The divergent directions of associations between the particle numbers and cholesterol concentrations of the HDL subclasses were apparent, but in the opposite directions to those in the VPA analysis. The effect sizes for these measures tended to be larger than in the VPA analysis, though not for the subclass triglycerides concentrations. Including waist circumference as an additional covariate in the models had a limited effect.

The associations between a 1 SD change in LPA (33.3 $min \cdot d^{-1}$) and the lipoprotein profile tended to be weak (Figure 17 and Supplementary table 18). Adjustment for waist circumference had a negligible effect on these associations.

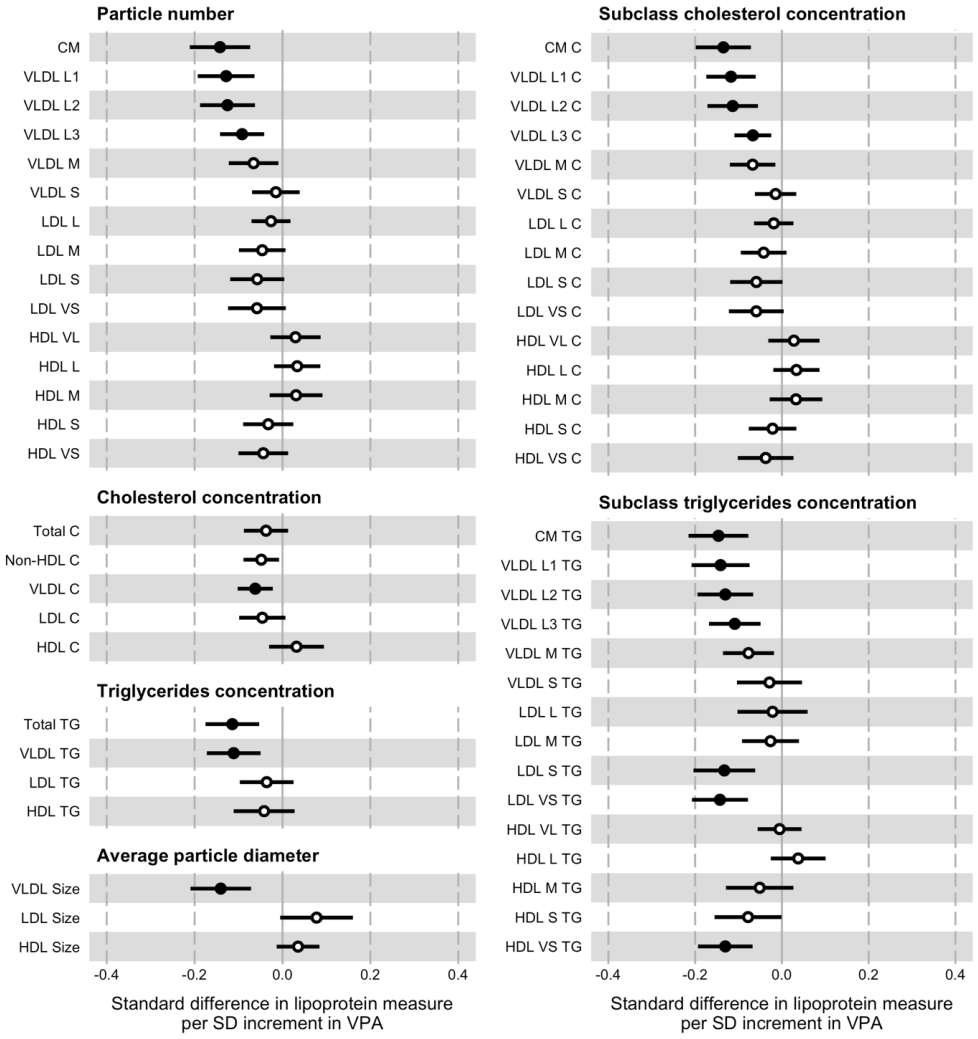
4.4.4. Associations between sedentary time and the lipoprotein profile

The associations between a 1 SD increment in sedentary time (57.5 $min \cdot d^{-1}$) were typically stronger with the VLDL particle measures (Figure 18 and Supplementary table 19). The directions of associations with all but one of these measures were the opposite of those in the prospective analysis of VPA (e.g., 1.22 x 10^{-1} $nmol \cdot L^{-1}$ or 0.12 SD; CI = 0.03, 0.21; $p = 0.01$ for VLDL L1 particle number). Though the effect sizes were smaller, the pattern of effect sizes decreasing from the largest to the smallest particles was replicated. For the HDL subclasses, the directions of associations with measures of particle numbers and cholesterol concentrations tended to differ dependent on the particle size, though the majority of individual effects were small to medium. The effect sizes for the triglycerides concentrations of the two subclasses of the smallest LDL particles were again more pronounced compared to other LDL subclass measures, which were typically small to null. The effect sizes of the associations for the triglycerides concentrations of the subclasses of the largest and smallest HDL particles were

relatively large compared to the other HDL subclasses. The degree of attenuation for individual measures having included waist circumference in the model were generally small.

The directions of association between a 1 SD change in sedentary time (52.8 min·d^{-1}) and the lipoprotein measures tended to be the opposite of those in the MPA change model, and the effect sizes smaller (Figure 19 and Supplementary table 20). Adjustment for waist circumference had a negligible effect on these associations.

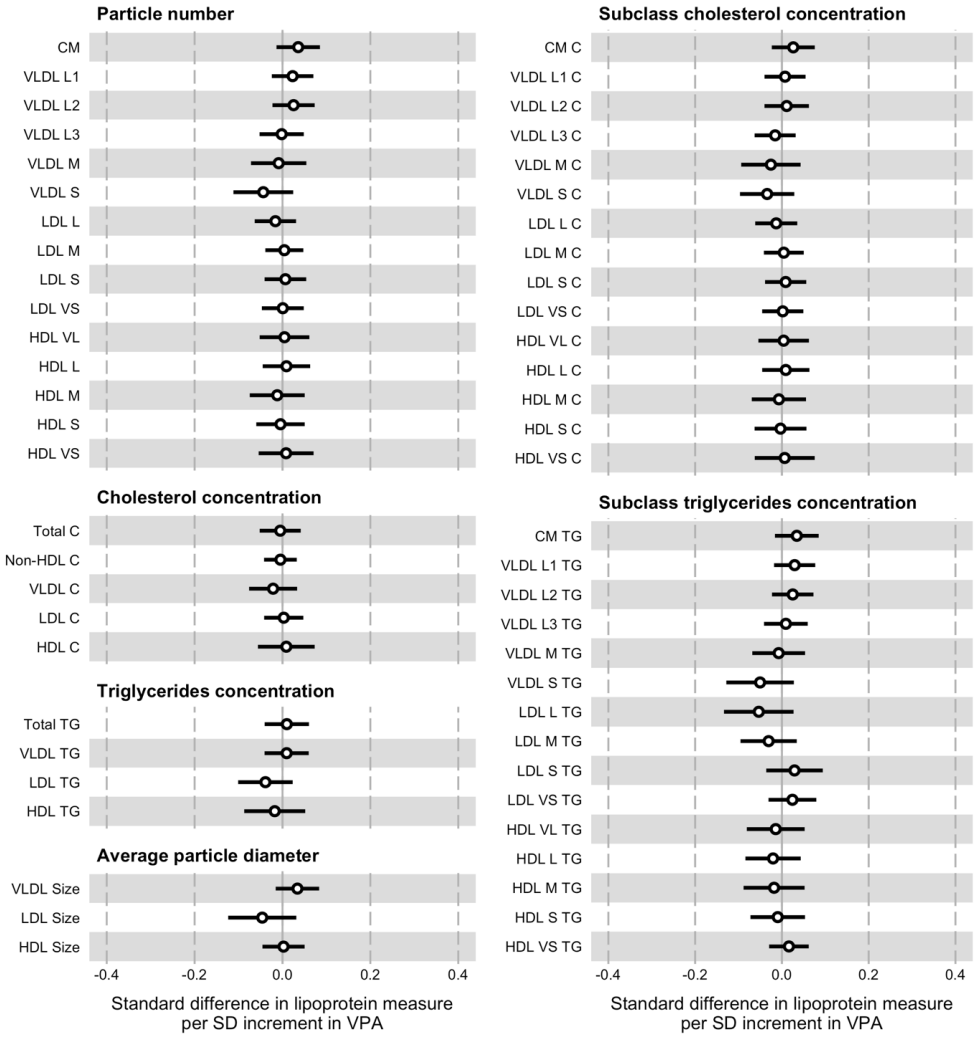
Figure 12. Associations between baseline VPA and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

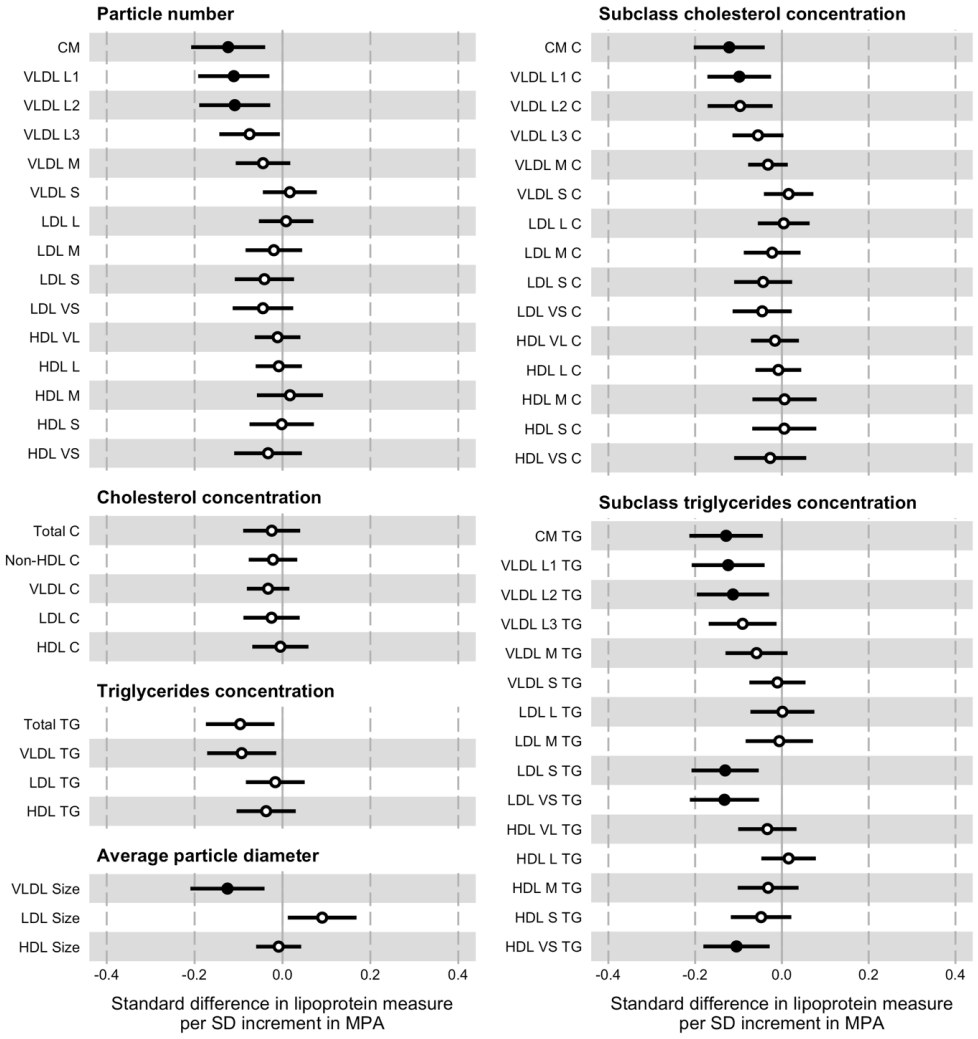
Figure 13. Associations between change in VPA and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

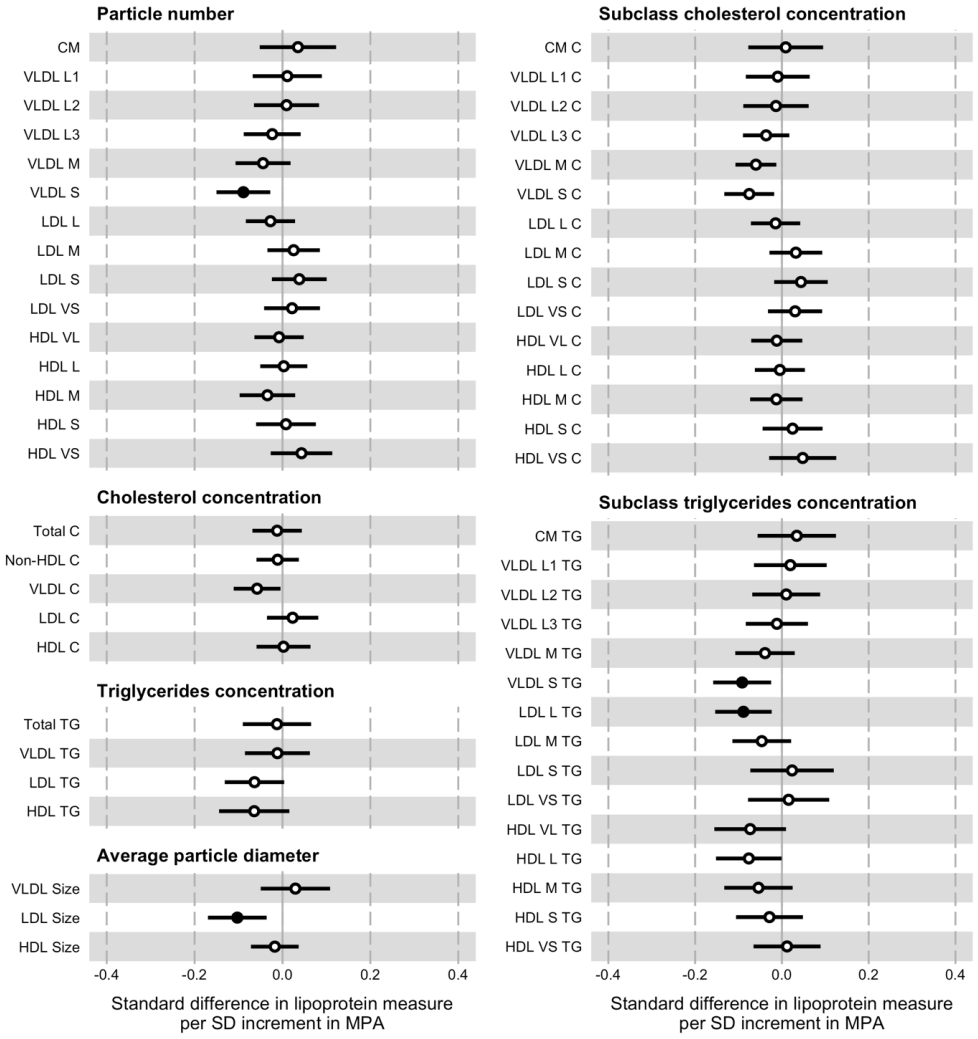
Figure 14. Associations between baseline MPA and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

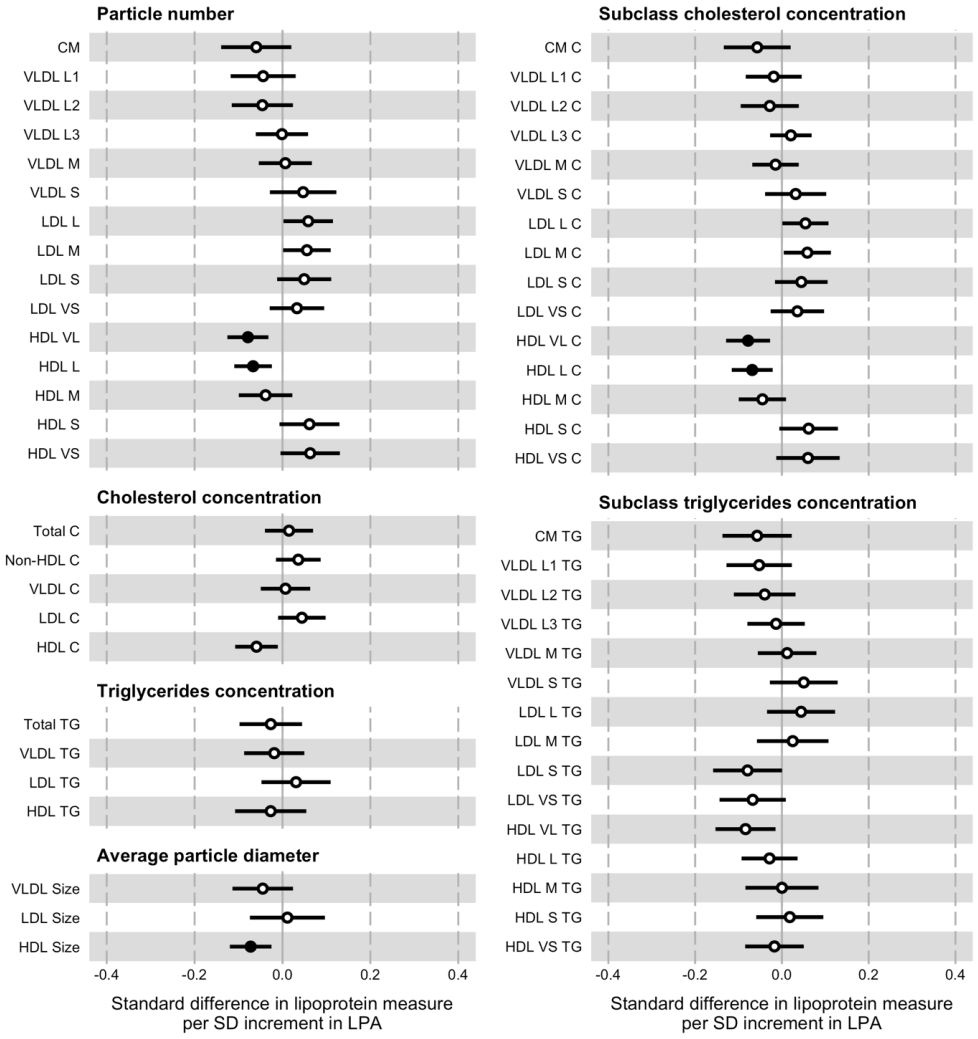
Figure 15. Associations between change in MPA and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

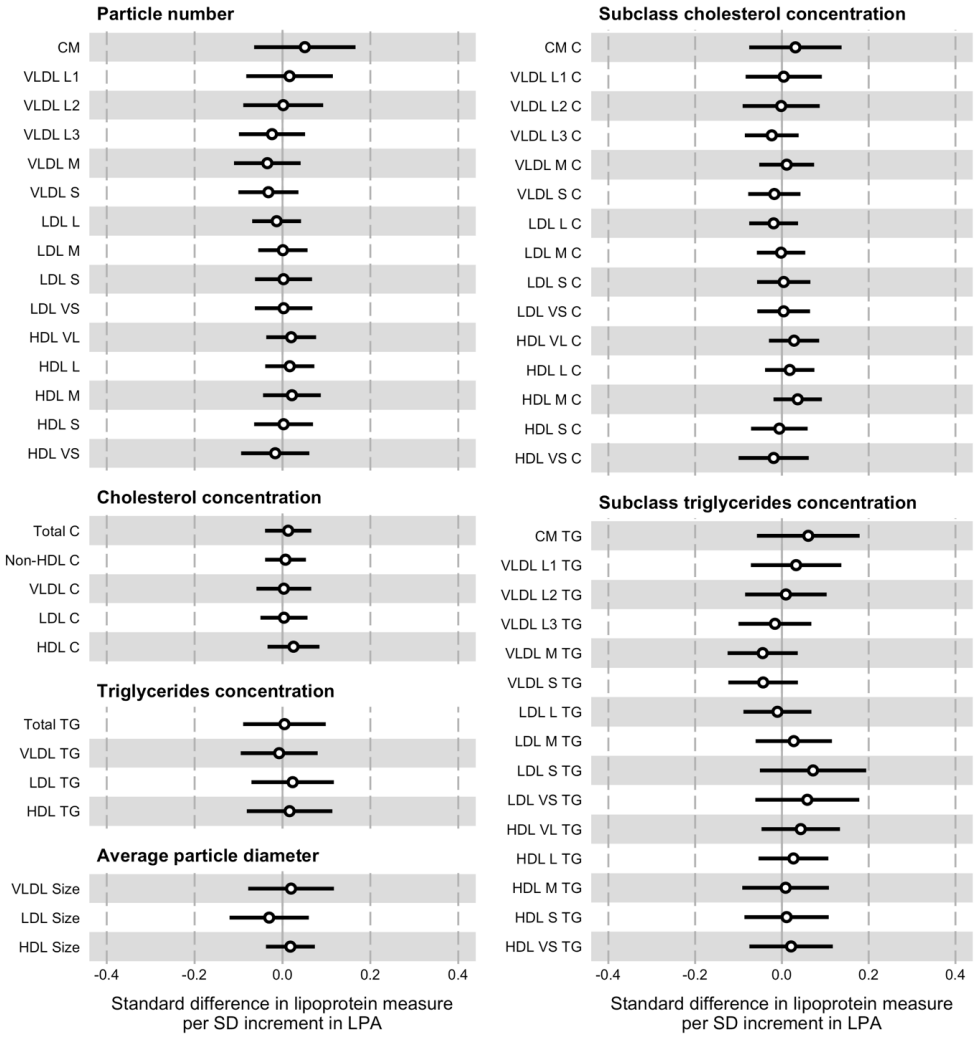
Figure 16. Associations between baseline LPA and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

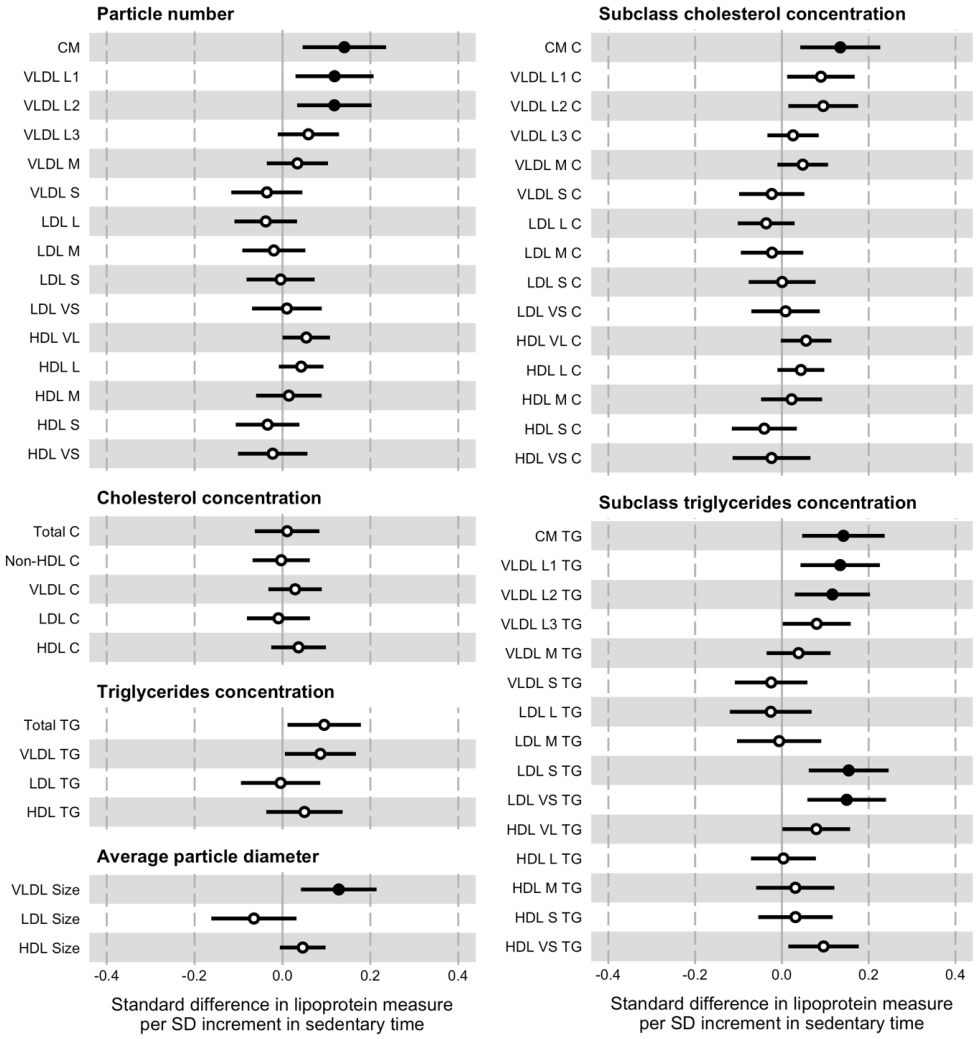
Figure 17. Associations between change in LPA and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

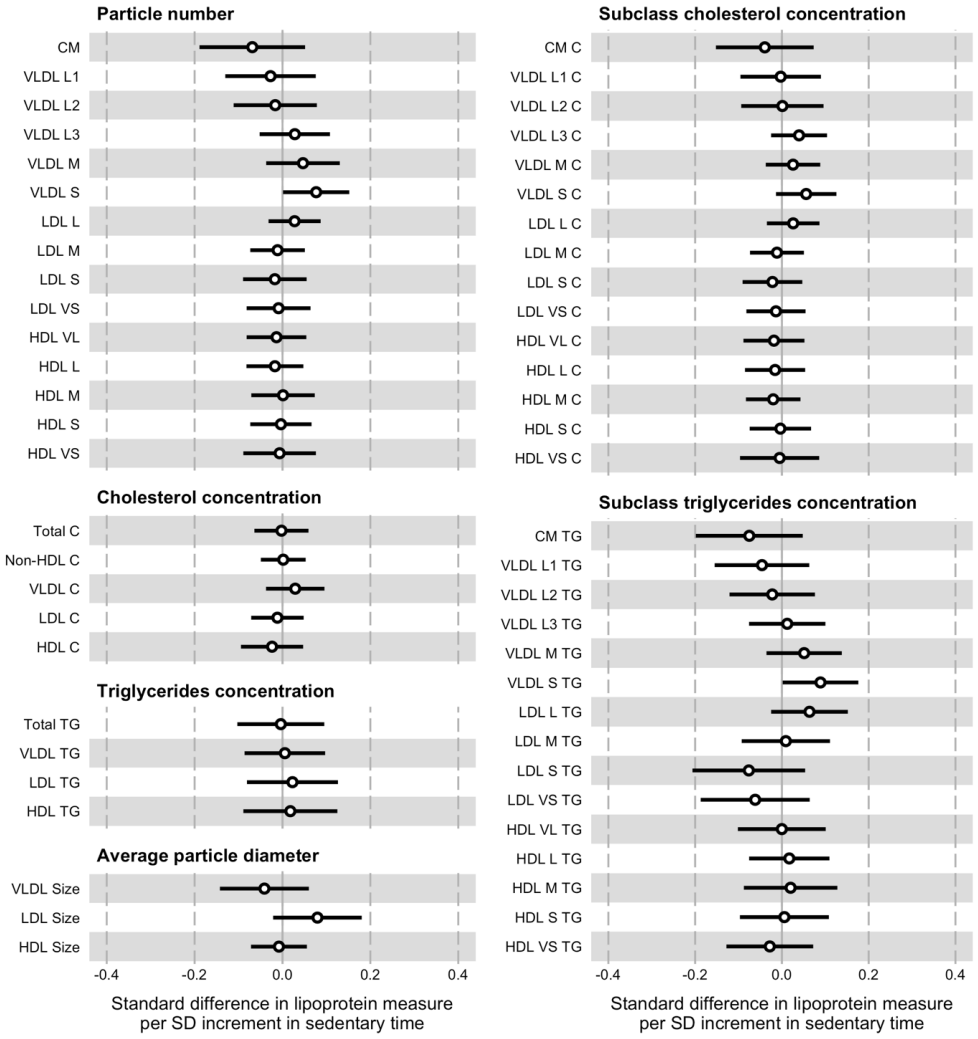
Figure 18. Associations between baseline sedentary time and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 19. Associations between change in sedentary time and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

4.5. Paper IV

4.5.1. Moderating role of daily moderate- to vigorous-intensity physical activity on the associations between aerobic fitness and lipoprotein subclass particle numbers

Aerobic fitness was prospectively associated with particle numbers of the five subclasses of the largest VLDL particles, independent of daily MVPA. Having included an interaction term in the models, a possible moderating role of daily MVPA was suggested for the three subclasses of the largest particles (Table 2).

Examining the trends for the associations between aerobic fitness and particle numbers at three different levels of daily MVPA (sample mean, sample mean ± 1 SD) indicated potentially differing effects of aerobic fitness for the five subclasses of the largest particles (Figure 20). At daily MVPA levels at or below the sample mean there were pronounced inverse associations, whereas the trends were negligible at levels above the mean. The trends did not differ greatly between the three levels of daily MVPA for the other subclasses.

At a level of aerobic fitness 1 SD below the mean, there were marked differences in the predicted values for subclass particle numbers between two levels of daily MVPA (sample mean ± 1 SD) for the three subclasses of the largest particles (Table 3). For these subclasses, predicted particle numbers were substantially higher at the lower level of daily MVPA. The estimates for the predicted values of the VLDL L3 subclass, and to a lesser extent VLDL M subclass, were suggestive of a difference, but the test statistic for the contrast was above the alpha threshold.

Table 2. Prospective associations between aerobic fitness and lipoprotein subclass particle numbers

Measure[a]	Term	Coefficient	Lower CI	Upper CI	*p* value
CM PN	Main effect	–0.134	–0.225	–0.042	0.005
	Fitness x MVPA	0.098	0.043	0.153	0.001
VLDL L1 PN	Main effect	–0.141	–0.229	–0.053	0.002
	Fitness x MVPA	0.096	0.039	0.152	0.001
VLDL L2 PN	Main effect	–0.143	–0.229	–0.056	0.002
	Fitness x MVPA	0.094	0.035	0.153	0.002
VLDL L3 PN	Main effect	–0.121	–0.195	–0.047	0.002
	Fitness x MVPA	0.057	0.003	0.110	0.037
VLDL M PN	Main effect	–0.113	–0.179	–0.047	0.001
	Fitness x MVPA	0.052	0.004	0.100	0.035
VLDL S PN	Main effect	–0.048	–0.127	0.030	0.224
	Fitness x MVPA	0.014	–0.035	0.063	0.558
LDL L PN	Main effect	–0.020	–0.087	0.047	0.549
	Fitness x MVPA	–0.010	–0.060	0.040	0.687
LDL M PN	Main effect	–0.019	–0.073	0.034	0.474
	Fitness x MVPA	–0.017	–0.062	0.028	0.455
LDL S PN	Main effect	–0.029	–0.082	0.025	0.288
	Fitness x MVPA	–0.015	–0.057	0.027	0.480
LDL VS PN	Main effect	–0.033	–0.087	0.021	0.220
	Fitness x MVPA	–0.016	–0.062	0.030	0.486
HDL VL PN	Main effect	0.004	–0.047	0.055	0.887
	Fitness x MVPA	–0.015	–0.062	0.032	0.515
HDL L PN	Main effect	0.002	–0.047	0.050	0.951
	Fitness x MVPA	–0.016	–0.062	0.030	0.484
HDL M PN	Main effect	0.016	–0.043	0.075	0.581
	Fitness x MVPA	0.000	–0.065	0.065	0.999
HDL S PN	Main effect	–0.042	–0.113	0.030	0.251
	Fitness x MVPA	0.047	–0.010	0.104	0.108
HDL VS PN	Main effect	–0.054	–0.122	0.013	0.113
	Fitness x MVPA	0.026	–0.031	0.083	0.370

Adjusted for baseline age, daily MVPA, parents' education, sex, sexual maturity, and subclass particle number. An interaction term between aerobic fitness and MVPA was included. Cluster-robust standard errors were calculated, clustered on the school variable.

[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.

Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

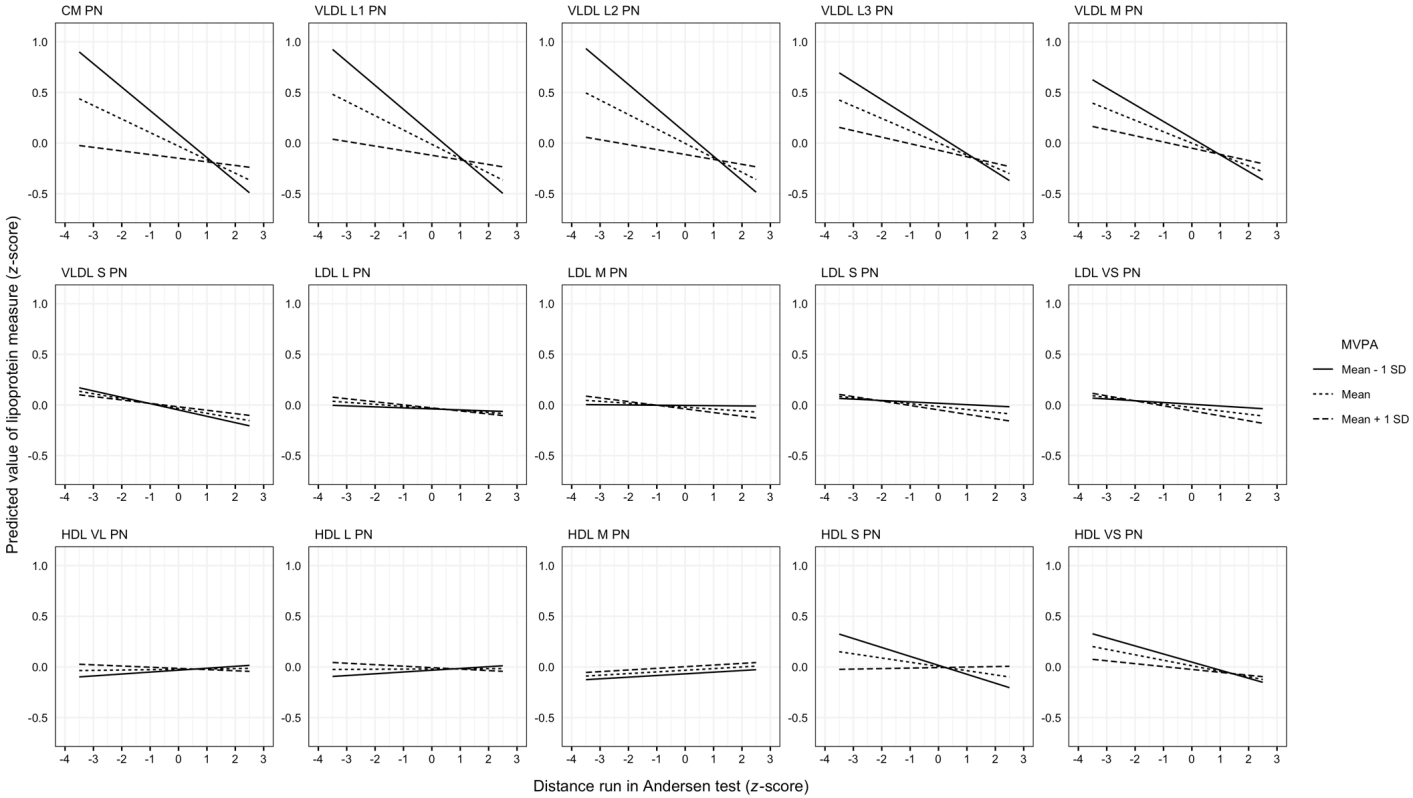
Table 3. Pairwise contrasts of predicted mean values of lipoprotein subclass particle numbers at Mean ± 1 SD aerobic fitness

Measure[a]	MVPA level	Estimate	Lower CI	Upper CI	Contrast	*p* value
CM PN	Mean – 1 SD	0.321	0.135	0.506	0.434	<0.001
	Mean + 1 SD	–0.114	–0.285	0.057		
VLDL L1 PN	Mean – 1 SD	0.333	0.145	0.521	0.408	<0.001
	Mean + 1 SD	–0.075	–0.240	0.090		
VLDL L2 PN	Mean – 1 SD	0.342	0.152	0.533	0.407	0.001
	Mean + 1 SD	–0.064	–0.236	0.108		
VLDL L3 PN	Mean – 1 SD	0.251	0.093	0.408	0.257	0.015
	Mean + 1 SD	–0.006	–0.168	0.155		
VLDL M PN	Mean – 1 SD	0.213	0.065	0.361	0.202	0.051
	Mean + 1 SD	0.011	–0.140	0.162		
VLDL S PN	Mean – 1 SD	0.013	–0.111	0.138	–0.002	0.977
	Mean + 1 SD	0.016	–0.133	0.164		
LDL L PN	Mean – 1 SD	–0.029	–0.147	0.090	–0.030	0.649
	Mean + 1 SD	0.001	–0.112	0.115		
LDL M PN	Mean – 1 SD	–0.002	–0.127	0.122	0.000	0.997
	Mean + 1 SD	–0.003	–0.109	0.103		
LDL S PN	Mean – 1 SD	0.031	–0.091	0.152	0.035	0.659
	Mean + 1 SD	–0.005	–0.123	0.113		
LDL VS PN	Mean – 1 SD	0.025	–0.099	0.148	0.033	0.683
	Mean + 1 SD	–0.008	–0.128	0.111		
HDL VL PN	Mean – 1 SD	–0.051	–0.144	0.041	–0.048	0.492
	Mean + 1 SD	–0.003	–0.136	0.129		
HDL L PN	Mean – 1 SD	–0.050	–0.137	0.036	–0.058	0.380
	Mean + 1 SD	0.007	–0.130	0.144		
HDL M PN	Mean – 1 SD	–0.085	–0.201	0.032	–0.071	0.431
	Mean + 1 SD	–0.014	–0.170	0.142		
HDL S PN	Mean – 1 SD	0.104	–0.043	0.251	0.115	0.191
	Mean + 1 SD	–0.012	–0.194	0.170		
HDL VS PN	Mean – 1 SD	0.127	–0.002	0.257	0.124	0.196
	Mean + 1 SD	0.003	–0.181	0.188		

[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.

Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Figure 20. Interaction plots of aerobic fitness and lipoprotein subclass particle numbers at three levels of MVPA



Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

5. Discussion

This thesis sought to investigate cross-sectional and prospective associations of PA, time spent sedentary, and aerobic fitness with a detailed lipoprotein profile measured using targeted ^{1}H NMR spectroscopy. Other pertinent hypotheses examined included to what extent any identified associations were attenuated by adiposity? and whether daily MVPA moderated the prospective associations between aerobic fitness and lipoprotein subclass particle numbers? There follows a consideration of the results from the four thesis papers contextualised into the extant research literature.

5.1. What are the associations of objectively measured physical activity and sedentary time with the lipoprotein profile?

5.1.1. Moderate-intensity and vigorous-intensity physical activity

In the cross-sectional analysis, there are consistent inverse associations between MVPA and almost all measures of VLDL particles. This pattern of associations is replicated in the prospective analyses of VPA and MPA, which contribute additional information in that the associations with the cholesterol and triglycerides concentrations of the VLDL subclasses correspond to the associations with particle numbers. Given that the concentration of lipids carried by these particles will be proportional to the number of particles circulating, this finding is not unexpected. As indicated by the inverse association with average particle diameter, the effect sizes are larger with the subclasses of increasing particle size. The differences in effect sizes between the VPA and MPA analyses are minimal. Taken together, higher amounts of PA of at least moderate intensity are associated with less and smaller triglyceride-rich lipoproteins, and less lipids being carried by these particles. These results are in keeping with a number of studies in children and adolescents that have reported beneficial associations between

objectively measured MVPA and total triglycerides concentration.[104,111,115] Additionally, a prospective analysis in adolescents of objectively measured MVPA and a comprehensive metabolic profile found inverse associations with the cholesterol and triglycerides concentrations of all the VLDL subclasses measured.[158]

The associations of VPA and MPA with the LDL measures are more modest than those with the VLDLs. In contrast to the VLDL analyses, the effect sizes decrease with subclasses of increasing particle size—additionally evidenced by the positive association with average particle diameter—but generally the effect sizes are small to null and not robust. This pattern of associations—relatively stronger inverse associations between higher PA and VLDL subclasses compared to LDL subclasses—has been reported previously in a study of adults.[141]

The associations of VPA and MPA with the measures of HDL subclass particle numbers and cholesterol concentrations are weak. Evidence of an association between MVPA and HDL-C concentration in children is inconsistent.[111,159] One possible reason for this is that the directions of associations with HDL subclass cholesterol concentrations seem to differ dependent on particle size, which would be obscured if using a single measure of HDL-C concentration.[141] Furthermore, the effect of PA to raise HDL-C concentration may be dependent on lower initial levels of HDL-C.[160,161] Given that our cohort were healthy and highly active at baseline, the variability in PA levels to observe a marked association is likely less than required. The inverse associations between the triglycerides concentrations of the two subclasses of the smallest HDL particles are relatively stronger than with the other HDL measures. This could reflect lower overall triglycerides concentration in more active children, or differences in HDL functionality, though this has not been explored. However, the proportion of circulating triglycerides transported by HDL is low and therefore whether these differences are of importance is difficult to say.[54]

The majority of associations between change in VPA and the lipoprotein measures are negligible to null. In the analysis of change in MPA, there are some more pronounced associations which tend to be with the VLDL S and larger LDL classes and LDL size. Given that the mean change in MPA between the two measurement occasions is inverse, the associations represent a decrease in these measures associated with an increase in MPA. A possible explanation could involve CETP. In adults, there is some evidence that prolonged exercise training results in a reduction in plasma CETP concentration, whereas higher CETP concentration is causally associated with higher concentrations of a number of measures of smaller VLDL subclasses, quantified using NMR spectroscopy.[162,163] Hence, an increase in MPA might be expected to be associated with lower measures of the smaller VLDL subclasses. This is speculative, however, and cannot explain why these associations are more pronounced in the MPA analysis than those in the VPA change analysis since the mean changes in both activity intensities were very similar.

5.1.2. Light-intensity physical activity

In both cross-sectional and prospective analyses, the associations between LPA and the lipoprotein profile tend to be more modest. Light-intensity physical activity is less strongly associated with VLDL measures than either VPA or MPA and, given the change in direction between some consecutively sized subclasses these associations are likely less robust. The lack of association may be because PA of lower intensities may not induce the energy deficit required to stimulate a reduction in circulating triglycerides. It could also be due to misclassification of some sedentary activities as LPA, which has previously been reported and would reduce the strength of any associations.[126]

Light-intensity physical activity is positively associated with most measures of LDLs. This is unexpected given that PA, if it at all affects LDL particles, is thought to benefit LDL

metabolism by lowering particle numbers or LDL-C concentration as a result of increased EE. Though an effect on these particles may only be achievable with more intense PA or in those who have a comorbid condition that is known to affect lipoprotein metabolism, such as obesity.[160,164] Again, these may be spurious associations due to misclassification of sedentary time as LPA, or potentially confounded due to those children who spend more time in LPA spending less time in MPA or VPA and are therefore insufficiently active at the higher PA intensities to benefit LDL metabolism. Interestingly, the relatively stronger, inverse associations with the triglycerides concentrations of the LDL M and LDL S subclasses are present and in a consistent direction to the VPA and MPA analysis. Given that the effect sizes are small and contradict the existing literature, which has shown that LPA has minimal effect on LDL-C concentration, these results should be interpreted with caution.[165,166] They require replication and further investigation.

The associations with the HDL measures are similarly unexpected. Though the divergent pattern of associations between subclasses of larger particles compared to those of smaller particles remain, the directions tend to be the opposite of those in the VPA and MPA analyses and the effect sizes slightly larger. This is reflected by an association with smaller average particle diameter and lower level of HDL-C concentration. Again, these findings conflict with the existing literature which suggests that higher levels of PA (including LPA), if at all they are associated with HDL-C concentration, are associated with higher levels of HDL-C.[165]

The majority of associations in the analysis of change in LPA are negligible and not robust.

5.1.3. Sedentary time

The individual cross-sectional and prospective associations between higher levels of time spent sedentary and the VLDL measures are typically the opposite of those in the analysis of VPA, and the effect sizes smaller. This demonstrates a potentially detrimental effect of higher levels

of time spent sedentary on triglycerides metabolism. However, it is uncertain how higher levels of sedentary time would induce an increase in VLDL secretion independent of energy balance due to lower amounts of PA or increased dietary intake. Instead, it is possible that these results reflect inactivity; that those children with higher levels of time spent sedentary are physically active less frequently, at lower intensities, or accumulated less PA volume. This is supported by the results of the isotemporal substitution analysis, which indicate that replacing sedentary time with an equal amount of MVPA would theoretically be beneficial for VLDL metabolism to the same extent as in the single activity MVPA model. Similar theoretical effects on total triglycerides concentration were reported in other studies that modelled isotemporal substitutions in both children and adults and suggest that the effect of sedentary time is not independent of PA.[126,167]

This is also likely the case for the associations between sedentary time and the LDL measures, which tend to be negligible and those that aren't are in the opposite directions to and weaker than in the VPA analysis.

That the associations with the HDL measures are generally in the same direction as in the VPA analysis is unexpected. Positive associations with the particle numbers, cholesterol concentrations, and to a lesser extent triglycerides concentrations, and shift to larger HDL size and HDL-C concentration contradict the results seen in another study which examined associations between objectively measured sedentary time and a comprehensive metabolic profile in adolescents.[158] These associations could reflect functional heterogeneity of HDL subclasses either dependent on or independent of PA.

The associations in the analysis of change in sedentary time between the two measurement occasions are again in opposite directions to those in the VPA and MPA change analyses, and probably represent the effects of a reduction in MVPA between the two measurement occasions. However, the effect sizes are small and not robust.

5.2. What are the associations of aerobic fitness with the lipoprotein profile and does moderate- to vigorous-intensity physical activity moderate them?

There are substantial associations between aerobic fitness and almost all lipoprotein measures in the cross-sectional analysis. The associations with the LDL subclass particle measures also seem consistent with those of the VLDL subclasses. In the prospective analysis, however, the degree of attenuation is greater for the LDL and HDL particle measures, such that the overall pattern of associations more closely resembles that of the prospective analysis of VPA. Though other studies have reported associations between aerobic fitness and measures of VLDL subclasses, a mechanism by which higher levels of aerobic fitness might influence either the production or clearance of these particles independent of PA is uncertain.[140,168] Indeed, improved fitness subsequent to three months of running training does not appear to influence the rise in triglyceride-rich lipoproteins observed after eating—postprandial lipaemia (PPL)—beyond the acute effects of a single exercise bout.[169] Hence, the attenuation of associations in the prospective analysis suggests that the cross-sectional associations with LDLs and HDLs are more dependent on their baseline levels; the attenuation reflecting that these measures are typically more stable and less likely affected by the repeated acute exposures to PA than those of VLDL. In fact, the interaction analysis indicates that the inverse associations between aerobic fitness and the particle numbers of the VLDL subclasses are driven by the amount of daily MVPA. Those children attaining a higher level of MVPA show negligible associations for aerobic fitness, which suggests energy expenditure or balance is the important factor, though the volume of MVPA at which this occurred is above current guideline recommendations.

Furthermore, the strong associations between aerobic fitness and individual lipoprotein measures in both the cross-sectional and prospective analyses are greatly attenuated when waist

circumference is included in the models. This finding is replicated in the interaction analysis, whereby including waist circumference in the models of aerobic fitness adjusted for daily MVPA results in null associations for all measures. Given the strong inverse associations between waist circumference and aerobic fitness, waist circumference likely confounds the associations between aerobic fitness and the lipoprotein profile. One potential reason for the substantial attenuation due to waist circumference is that field tests such as the Andersen test are performance-based and do not directly measure $\dot{V}O_{2peak}$. They are also likely influenced more by anthropometric and psychological factors such as adiposity, which is problematic since adipose tissue is metabolically inert and should not influence $\dot{V}O_{2max}$.[170] Another study that measured lipoprotein subclasses using NMR spectroscopy reported a similar pattern of associations between levels of aerobic fitness—measured using a graded cycle ergometer test until exhaustion and predicted $\dot{V}O_{2max}$ estimated from HR and maximal power—and lipoprotein profile, and that a number of individual associations remained after adjustment for body fat percentage.[140] Hence, the associations adjusted for waist circumference reported in this thesis may be overly biased towards the null as a result of the less precise measure of aerobic fitness.

5.3. What are the associations between adiposity and the lipoprotein profile?

The associations of adiposity and the various measures of the lipoprotein profile are stronger in magnitude than in the analyses of aerobic fitness or any of the PA variables. Similar to the analyses of aerobic fitness, and PA and sedentary time, the associations with LDL and HDL measures are typically attenuated to a greater degree than the VLDL measures in the prospective compared to cross-sectional analysis. This likely reflects a greater stability of LDL and HDL measures over the short follow-up period such that adiposity has relatively less

influence than other innate determinants of their synthesis or clearance. The pattern of associations is comparable to that of a study which examined the relationship between BMI and lipoprotein subclass measures in adolescents using both multivariable linear regression and Mendelian randomisation with cross-sectional data.[171] A causal role for adiposity in raising triglyceride-rich VLDLs, small and medium LDLs, and lowering HDL-C concentration was reported and considered unlikely to be a result of residual confounding given the consistency of effects in both the cross-sectional and Mendelian randomisation analyses. This suggests that adiposity is likely causal for many of the individual associations reported in this thesis.

Of particular note in the prospective analysis of adiposity are the strong associations with triglycerides concentrations of the subclasses of larger VLDL particles, smaller LDL subclasses, and smaller HDL subclasses, and the increased average diameter of VLDL particles, and decreased average diameter of smaller LDL and HDL particles. This pattern of lipoprotein changes—triglyceride-enriched VLDLs, particularly the subclasses of larger particles, and smaller, triglyceride-enriched LDLs and HDLs—is commonly observed in syndromes and diseases associated with insulin resistance, such as MetS and T2DM.[172] Excessive adiposity, especially in a truncal/abdominal distribution, can result in the liver and peripheral tissues becoming resistant to insulin, with consequent increased rates of hepatic triglyceride-enriched VLDL secretion.[173,174] These larger, triglyceride-enriched VLDLs may transfer their triglycerides to both LDL and HDL particles, mediated by CETP, resulting in small, dense LDL particles, and triglyceride-enriched HDL particles.[172] Given that adiposity is causal for similar lipoprotein subclass changes as those described with insulin resistance, and that these causal associations are continuous and without a threshold whereby lower BMI has no effect on the lipoprotein profile, the results in this thesis indicate some potentially detrimental metabolic consequences of adiposity within the cohort.[171]

In the cross-sectional and prospective analyses of PA and sedentary time, including waist circumference as a covariate in the models led to attenuation of many of the associations with individual lipoprotein measures. Yet, the magnitudes of attenuation are small and the overall patterns of associations remained consistent with the analyses not adjusted for waist circumference, which indicates an independent association of PA and sedentary time on many of the individual measures. Attenuation for the VLDL measures in the prospective VPA analysis tend to be larger, which to some extent reflects the causal role for adiposity on these particles and that higher levels of adiposity may counteract the beneficial effects of intense PA or inhibit children with higher levels of adiposity engaging in VPA.

5.4. Biological plausibility

5.4.1. Apolipoprotein B-containing lipoproteins

The well-recognised causal effect of LDLs on ASCVD risk may not be due to their lipid mass but the concentration/number of particles in the circulation.[59,75,175,176] Evidence of this was demonstrated by a study that used Mendelian randomisation to compare risk of CHD between individuals with LDL-C concentration lowering variants of the low-density lipoprotein receptor (LDLR) gene and those with triglyceride-lowering variants of the LPL gene.[78] The authors reported that the clinical benefit of lower triglycerides on CHD risk was similar to that of lower LDL-C concentration, and that the associations were independent of each other and proportional to the absolute change in ApoB-containing lipoproteins. This suggests that all ApoB-containing lipoproteins have the same effect on CHD risk per particle. Additionally, all ApoB-containing lipoproteins up to 70 nm diameter, which includes many VLDL particles and their remnants, can penetrate the arterial intima and therefore have equivalent atherogenic potential.[80] Since the circulating number of ApoB-containing particles likely determines the probability of them entering and being retained in the arterial intima, and the larger effect sizes

seen in this thesis are with VLDL particles, any cardioprotective effects of higher levels of PA are likely expressed through differences in the metabolism of these larger ApoB-containing lipoprotein subclasses.[66] It should be borne in mind that for normolipidaemic individuals in the fasting state, LDLs account for more than 90% of the circulating ApoB and that therefore VLDLs represent a relatively small proportion of the potential atherogenic burden.[177] However, in the non-fasting state, the burden is higher due to the presence of chylomicron and VLDL metabolic remnant lipoproteins, which are also considered atherogenic.[178]

Furthermore, atherosclerotic plaques grow over time due to the accumulation of retained lipoprotein particles. The size of total plaque burden at a given time is proportional to the circulating concentration of ApoB-containing lipoproteins and the total duration of exposure to these lipoproteins, and naturally increases with age.[179] Eventually, the plaque can reach a threshold size above which there is a risk of having a CVD event. Given that atherosclerosis starts in childhood and the majority rise in LDL-C concentration occurs prior to early adulthood, it seems intuitive that primordial prevention of suboptimal lipid levels should occur in childhood and adolescence. If the associations between PA and lower ApoB-containing particle numbers reported in this thesis are true effects, this suggests increased levels of PA could contribute to lower long-term CVD risk.

5.4.2. Physiological mechanisms

Very low-density lipoproteins are the primary carriers of triglycerides and triglycerides are the primary lipid component of VLDLs.[65] Hence, any effect of PA on VLDL particles will likely be due to an effect on triglycerides metabolism. Fatty acids, such as those produced by hydrolysis of triglycerides, are an important source of energy both at rest and whilst exercising. During exercise, the rate of fatty acid oxidation can increase ten-fold compared to resting levels.[180] Given the role of triglycerides as an energy source, it seems likely that higher levels

of PA would be associated with lower levels of circulating triglycerides as a result of higher EE. Although there is a reduction in the rate of VLDL triglycerides secretion during exercise, it is unlikely that the lower concentration of circulating particles is due to increased use of the triglycerides being transported by VLDLs as substrate for lipid oxidation *during* exercise.[181] However, an increased rate of VLDL clearance *subsequent* to a bout of aerobic exercise has been demonstrated.[182] In addition, it appears that this exercise-induced reduction in circulating triglycerides concentration is short-lived, and typically abolished within 48 hours after the exercise bout.[183] Thus, in this thesis, the prospective associations with lower levels of VLDL measures probably reflect the acute effects of consistent repeated engagement with PA, hence exercise-induced reduction in triglycerides concentration across the follow-up period, as opposed to more permanent metabolic adaptation to chronic PA behaviour.

Given that LDL particles are a result of the delipidation of VLDL particles, it might be expected that the inverse associations between higher levels of PA and LDL particle numbers would be equal to the associations with VLDL particle numbers. However, the stimulatory effect of exercise on VLDL delipidation does not appear to stimulate the subsequent conversion of IDL to LDL.[182] Furthermore, smaller VLDL particles can be cleared directly from blood plasma by the liver, though the determinants for why one outcome may predominate compared to the other are currently unknown.[175] In both the VPA and MPA analyses, there are two distinct inverse associations with the triglycerides concentrations of the two LDL subclasses of the smallest particles. There are pathophysiological reasons for differences in triglyceride accumulation in LDL subclasses typically associated with adiposity and/or insulin resistance.[174] However, the associations with the triglycerides concentrations of these two subclasses are apparently independent of waist circumference, which could indicate an independent benefit of PA to overall triglycerides clearance. That the effect size of the association with total LDL triglycerides is more consistent with the smaller effect sizes of the

LDL L and LDL M subclasses suggests that the associations with absolute differences in LDL triglycerides levels are modest. Considering only a small proportion of circulating triglycerides are carried in the LDLs, whether this effect provides an appreciable benefit in a young, healthy cohort is uncertain, but if sustained into adulthood where dyslipidaemia and other metabolic aberrations tend to manifest, it could be of clinical importance.

5.4.3. High-density lipoproteins

Substantial structural, compositional, and functional heterogeneity exists between HDL particles. In addition to RCT, HDLs participate in a diverse assortment of biological functions which seem to be mediated by different particle subpopulations.[62] In the prospective analyses, particle numbers and cholesterol concentrations of the HDL subclasses are directionally consistent between VPA and sedentary time, suggesting a shift towards larger HDL particles and increased serum HDL-C concentration. However, the associations are weak in both analyses and negligible in the MPA model, so may not be true effects. If true, it is challenging to provide a mechanistic explanation for this apparent paradox contingent on energy expenditure alone, especially given that the associations are in the reverse direction in the LPA analysis. Instead, these results may reflect the poor characterisation by particle numbers or lipid mass of HDL physicochemical and functional heterogeneity, and the incomplete understanding of how HDL function changes with either total PA or PA of different intensities.[62] Historically, that higher HDL-C levels are associated with higher levels of PA and lower CVD indicated a potential means through which PA exerted its cardioprotective effect.[61] However, results from Mendelian randomisation and clinical trials in which HDL-C concentration was increased significantly with pharmacotherapy but failed to result in a concomitant reduction in CVD event rate compared to placebo, indicate that a direct causal effect of HDL-C level on CVD is unlikely.[76,81,82,184] Furthermore, there is preliminary evidence that exercise benefits some HDL

attributes independent of changes in HDL-C concentration.[185] Consequently, greater research effort has been directed to quantifying HDL functionality, its influence on CVD risk, and the effects of PA of different intensities on HDL beyond the traditional lipid profile.[186,187] The generally modest associations with HDL measures reported in this thesis suggest the cardioprotective effects of PA are either inadequately characterised by measures of particle numbers and lipid load, or alternatively, indicative of limited metabolic perturbation in a young, healthy cohort.

5.5. Methodological considerations

5.5.1. Participants

Recruitment

Recruitment to the ASK study was very successful: 1129 children underwent baseline testing, which was 94.0% of 1202 children deemed eligible for inclusion and 80.9% of fifth grade children in Sogn and Fjordane county. Though dropouts from cohort studies are usually substantial, retention of children within the ASK study randomised controlled trial was high.[142] As a cohort, the sample is therefore representative of the original population of interest. The analytical sample for each paper does vary due to availability of valid data for individuals but does not differ from the group not analysed with regard to the traditional clinical chemistry measures of lipid metabolism in any of the papers. There are differences between the analysed and non-analysed groups for certain predictors—PA in Paper III and distance run in the Andersen test for Paper IV—but given that the standard lipid profile measures don't differ between groups at baseline, the possibly that this introduced bias is low. It is also unlikely that the groups not analysed are less healthy since a lower proportion are overweight or obese in Papers II and III, and the entire cohort young and healthy at recruitment. However, despite the prevalence of overweight and obesity in the ASK cohort being similar to children in other

European nations, they are atypical in that they are highly active.[188,189] Therefore, the use of sample-estimated levels of PA and sedentary time may limit the generalisability of results.

Data collection

There was a high degree of compliance with data collection. Of the children that underwent baseline testing, 1006 (89.1%) and 820 (72.6%) recorded ≥4 and ≥6 days of valid accelerometer data, respectively. The mean accelerometer wear time was 778 $min \cdot d^{-1}$. A high proportion—936 children (82.3%)—participated in aerobic fitness testing. The collection of blood samples at two time points enabled investigation of the temporal sequence of associations between exposures and the lipoprotein profile, which affords greater confidence in the resultant associations than cross-sectional studies. The high compliance with testing permitted adjustment for a number of putative confounders and to examine interactions between two of the main exposures. Still, given that the studies are observational, the possibility that the reported associations are biased due to unmeasured or poorly measured confounding cannot be excluded. For example, information on dietary intake, which undeniably affects lipoprotein metabolism, was not collected. Therefore, it is not possible to assess energy intake or consider the effect of positive energy balance on adiposity or how this might connect to PA. Furthermore, the use of fasted blood samples cannot capture the influence of dietary intake on lipoprotein metabolism, precluding the possibility of examining potentially beneficial associations of PA with chylomicron and VLDL metabolic remnants.[65]

5.5.2. Assessment methods

Physical activity and sedentary time

The use of accelerometers to measure PA and sedentary time is an asset. Device-based measures typically exhibit lower variability than self-report methods when compared to DLW

and are less prone to the subjective biases of participants. These measures are more comparable across populations, especially when using similar accelerometer models and data-reduction protocols.[190] Though certainly desirable, it was not feasible to supply the participating children with accelerometers to wear throughout the study period, so a 7-d measurement period is relied upon as being representative of habitual PA behaviour. However, considerable intraindividual variation in children's PA across a year has been reported, such that regression coefficients using a single assessment of seven consecutive days of accelerometer data may underestimate true associations by as much as 50%.[191] Use of 10-s epoch enabled capture of the typically sporadic PA pattern observed in children.[192,193] Unfortunately, accelerometers that are hip-worn are poor at capturing energy expenditure from cycling, load-bearing activities, walking on inclines, and cannot be worn during activities that involve water immersion, such as swimming.[194] Consequently, PA measurement in children that participate in these activities will have been underestimated and consequently the effect sizes attenuated due to a reduction in the variability of PA exposure. However, it is impossible to determine the extent to which this affected the ASK cohort or whether there were disparate effects between groups with more or less favourable lipoprotein profiles. Given the generally high levels of PA across the cohort, this issue may be immaterial.

Aerobic fitness

There is ongoing debate regarding whether field tests of performance are valid measures of aerobic fitness.[49] A criticism of such tests is that they are influenced by body weight and penalise heavier individuals. Given the observation that obese individuals tend to have similar $\dot{V}O_{2max}$ to normal weight individuals after normalising for differences in body size, a test of aerobic fitness that penalises heavier individuals cannot be said to be testing maximum oxygen consumption.[170] The same reasoning underlies the critique of scaling $\dot{V}O_{2max}$ or $\dot{V}O_{2peak}$ by

body mass, as opposed to fat-free mass. Rather than $\dot{V}O_{2max}$, it seems that individuals with higher levels of adiposity are limited in their sub-maximal aerobic capacity when participating in weight-bearing activities. For example, it has been reported that obese individuals use a higher proportion of their $\dot{V}O_{2max}$ when walking, and that consequently such tasks are more difficult physiologically.[195] Field tests that involve running could perhaps better be described as tests of aerobic performance. Notwithstanding the influence of anthropometrics, the Andersen test has been reported as being both a valid and reliable tool for assessing aerobic fitness in school-aged children at the group level and associated with markers of metabolic health.[52,196] Using the Andersen test as a measure of aerobic fitness to explore associations with lipoprotein attributes without attempting to provide mechanistic explanations regarding maximum oxygen consumption therefore seems reasonable. However, it is important to determine which elements that contribute to performance in the Andersen test do influence lipoprotein metabolism or whether the associations are mainly (or wholly) a result of adiposity.

5.5.3. Analytical methods

Accelerometer wear time criteria

Though the wear time criterion for a valid day of accelerometer data is lower than many studies (≥480 min·d^{-1}), the reliability of PA measurements at the group-level is likely high in the two papers that modelled PA as a primary exposure. In Paper III, the sample is restricted to children with ≥4 days of valid data, which has shown acceptable reliability using a minimum wear time of 480 min·d^{-1}.[197] In Paper I, though all children with ≥1 day of valid data are included, the change in associations is negligible in the sensitivity analysis where inclusion was restricted to those children with ≥4 days valid data. The non-wear criterion of at least 20 minutes of consecutive zero counts has previously been reported as the most appropriate when assessing sedentary time objectively using accelerometers.[198] However, accelerometers cannot classify

activities performed during sedentary time, such as television viewing, which has been shown to predict more detrimental lipid levels in children over two years, independent of PA, aerobic fitness, and adiposity.[199]

Statistical methods

When performing individual regression analyses on numerous outcome variables, the possibility of reporting Type I errors is high. There are conflicting opinions regarding whether applying a correction for multiple testing is required in exploratory studies such as those in this thesis.[200] Though appropriate multiple comparison adjustments are applied—either FDR correction or Bonferroni adjustment—and the resulting *p* values reported, these tests should be interpreted in conjunction with the respective effect sizes and 95% confidence intervals.[201,202] The results should be tested in confirmatory studies whereby the hypotheses are specified *a priori*.

5.5.4. Inferences

Lipoprotein profile

Phenotyping using a targeted metabolomics platform permitted investigation of the associations of PA, time spent sedentary, and aerobic fitness with a variety of lipoprotein attributes. These novel insights deepen knowledge of the biology that underpins the metabolic benefits of higher levels of PA and aerobic fitness and suggest interesting associations that require closer examination. However, many of the measures are collinear—more than 95% of the variance was explained by five principal components—which indicates that the measured attributes are likely signifiers of the same continuous metabolic processes—such as VLDL delipidation—or of heterogeneous particle subpopulations—such as HDLs—rather than being direct, independent measures of particle function. The fine-grained lipoprotein phenotypic

information obtained by NMR spectroscopy should be complemented with measures of function—for example, HDL subspeciation information from proteomic analysis or kinetic studies of RCT and cholesterol efflux—to provide a more comprehensive, integrated mechanistic understanding of these biological processes.[203]

Causation

Since the children that participated in the ASK study were young and the period over which they were followed short, variation in the measures of lipoprotein metabolism is likely low and will have biased associations towards the null. The short follow-up period and measurement at two time points limited the scope for change in the variables of interest, which likely contributed to the negligible associations in the change models in Paper III. Also, though there are effects of exposure variables independent of adiposity, the short follow-up time tempers assigning causation. Given that adiposity is causal for many of the lipoprotein measures, and there is bidirectional causation between adiposity and PA, the direction of causation between PA and the lipoprotein measures cannot be assumed.[118,171] It would be instructive for studies to attempt to replicate and validate the findings in larger, more diverse populations with greater metabolic variability, over longer periods of time, to strengthen causal inference and determine whether the potentially favourable effects of PA, if sustained, are augmented with age and biological attrition.

5.6. Potential implications of findings

5.6.1. Relevance for public health

There is unequivocal evidence that higher levels of PA and aerobic fitness are beneficial for clinical outcomes, such as CVD and all-cause mortality.[37,86–88] Reducing time spent sedentary is also important.[37,85,89,90] Excess adiposity undoubtedly so.[204,205] Disentangling the respective

contributions of each determinant, though important, is a task almost Sisyphean in nature as they are neither completely intrinsic, nor do they exist in isolation, themselves being determined by behavioural, and hence also social, economic, and other extrinsic factors. What is true for these clinical endpoints appears to also be true for lipoprotein metabolism. Physical activity of at least moderate intensity is associated with a favourable lipoprotein profile, as is a replacement of time spent sedentary with MVPA. If a higher level of aerobic fitness does have an independent beneficial effect on the lipoprotein profile, improving aerobic fitness by increasing MVPA would be valuable. Engagement in higher levels of MVPA might also mitigate the detrimental associations with lower fitness. Hence, increasing levels of MVPA and/or replacing time spent sedentary would appear important to improve cardiometabolic health. Notwithstanding that the associations of PA and sedentary time with some lipoprotein measures appear to be independent of waist circumference, given the strong causal association between adiposity and the lipoprotein profile and causal association with lower total PA, MVPA, and increased time spent sedentary, a concomitant reduction in adiposity would likely be synergistic. Furthermore, since the effect of PA on the number of circulating ApoB-containing lipoproteins is likely to be acute and not maintained beyond a few days, as opposed to a sustained metabolic adaptation, any preventive programme or intervention requires frequent, repeated engagement with PA maintained across the life course. These suggestions are speculative, however, and caution is advised given that these data are observational. Replication and more robust causal and experimental evidence are required before discussion of interventions that benefit the lipoprotein profile is warranted. This is especially so given that the reported absolute effect sizes with any individual lipoprotein measure in this cohort are small, and that the relevance of these lipoprotein attributes to health is unclear.

5.6.2. Does a detailed lipoprotein profile provide useful information?

Metabolic phenotyping by NMR spectroscopy has been applied to a broad range of epidemiological studies, resulting in a variety of interesting findings, including: associations between lipoprotein subclasses, atherosclerosis and subsequent CVD risk;[206] differing associations with incident ischaemic stroke compared to incident haemorrhagic stroke;[84] remarkably similar association patterns between the effects of commencing HMG-CoA reductase (HMGCR) inhibitor (statin) therapy to a genetic proxy for HMGCR inhibition;[207] evidence that the associations between maternal BMI and offspring metabolic traits are likely due to shared familial factors as opposed to maternal overnutrition;[156] and even metabolites associated with all-cause mortality.[208] Insights such as these may identify novel biomarkers of diseases or exposures and potential therapeutic targets, improve understanding of pathological processes, and contribute to determining the contributions of hereditary compared to environmental factors.

Naturally, given the more numerous measures and attributes now available for analysis through metabolic phenotyping and the causal role of lipoproteins to ASCVD, it might also be expected that advanced lipoprotein testing should result in improved prediction of clinical disease risk. However, despite evidence for certain measures, such as LDL or HDL particle numbers, being more sensitive indicators of CVD risk, it remains to be seen whether they offer any additional clinical utility beyond the standard lipid profile.[209,210] For example, compared to conventional measures of lipids or immunoassay-measured apolipoproteins, CVD risk prediction with NMR-measured lipoprotein profiles was comparable but not superior.[211] Furthermore, although data-driven subgrouping of comprehensive lipoprotein subclass data using artificial intelligence (AI) provided more detailed information regarding how lipoprotein subclass metabolism relates to risk of CHD, this multivariate classification performed worse at predicting CHD risk than a univariate classification using just ApoB concentration.[212] Many

measures included in the comprehensive lipoprotein profile are highly correlated, and some measure essentially the same attribute as other techniques, such as LDL particle number and the immunoassay of ApoB-containing lipoproteins, so as single measures may not be appreciably more useful.[213] However, as time progresses and other considerations such as cost effectiveness and analytical performance change, the greater amounts of information available from platforms such as NMR spectroscopic analysis may lead to an increase in their application in clinical settings.[214]

5.6.3. Future research

Examination of a detailed lipoprotein profile through the application of ^{1}H NMR spectroscopy revealed a number of thought-provoking associations with PA, sedentary time, and aerobic fitness, which require further exploration, and generated new questions and hypotheses. For example, do the different directions of association between PA and particle numbers of the HDL subclasses represent differing effects on HDL subspecies? and what, if any, are the physiological consequences? Measuring circulating particles provides insight into lipoproteins in transit, and how the attributes and relative proportions of different subclasses might be influenced by certain behaviours. Yet lipid transport within the circulatory system is only one element of a dynamic metabolic process. It is important to augment this knowledge with other lines of enquiry, such as studies of lipid kinetics and functionality, to investigate mechanisms and better understand the implications of the associations reported in this thesis. It is also necessary to determine whether the associations are clinically significant. Fundamentally, it remains to be elucidated whether the associations of PA, sedentary time, or aerobic fitness with the lipoprotein profile are causal, or to what degree they are independent of other exposures, such as adiposity and diet. Answering these questions requires not just larger sample sizes with more precise, more frequent measurements over longer durations, but different, complimentary

methods of causal inference to enable the drawing of more robust conclusions and therefore contribute constructively to any resultant interventions or health policy.

6. Conclusions

Taken in their totality and in the context of the existing literature, some conclusions can be drawn from the research that comprises this thesis.

Higher levels of PA of at least moderate intensity are favourably associated with a variety of measures of lipoprotein metabolism. These benefits are more pronounced with the triglyceride-rich lipoprotein particles and measures of triglycerides concentrations and are likely a consequence of PA-induced acute energy deficit. That these associations were evident in prospective analyses suggests that the effects of VPA and MPA may be causal, though this requires further investigation. Although adiposity is a strong influence on lipoprotein metabolism, PA seems to have an independent effect on a number of individual measures. Change in PA of any intensity or sedentary time appear of limited influence on lipoprotein metabolism, at least across the short duration of follow-up in the ASK study.

Aerobic fitness also appears beneficial for the lipoprotein profile. Again, levels are prospectively associated with a number of individual lipoprotein measures and predominantly those of the triglyceride-rich particles and triglycerides concentrations. There is some suggestion that daily MVPA moderates the associations between aerobic fitness and particle numbers of the subclasses of the larger particles, especially at lower aerobic fitness levels. However, many of the associations between aerobic fitness and individual lipoprotein measures seem heavily influenced, possibly confounded, by adiposity, though this is likely overestimated given the use of a field test of physical performance. A mechanism by which aerobic fitness influences lipoprotein metabolism independent of other factors such as PA is unclear.

The associations between LPA and the lipoprotein profile tend to be weaker than those of VPA and MPA and not robust. The directions of the more pronounced associations are contrary to

what would be expected if they are driven by EE and may be due to misclassification of time spent sedentary as LPA.

More time spent sedentary seems detrimental to lipoprotein metabolism; the pattern of associations tending to be the inverse of that with VPA. However, rather than being a direct metabolic consequence of sedentary time per se, this is probably attributable, at least in part, to less time spent physically active and hence less EE. Replacing a period of sedentary time with an equal period of MVPA theoretically ameliorates the detrimental associations.

References

1 Roth GA, Abate D, Abate KH, *et al.* Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2018; **392**: 1736–88.

2 World Health Organization. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. Geneva: World Health Organization, 2013.

3 Shiroma EJ, Lee I-M. Can we proceed with physical activity recommendations if (almost) no clinical trial data exist on mortality? *Br J Sports Med* 2018; **52**: 888–9.

4 World Health Organization. Global recommendations on physical activity for health. Geneva: World Health Organization, 2010.

5 U.S. Department of Health and Human Services. Physical Activity and Health: A Report of the Surgeon General. Atlanta, G.A.: U.S. Department of Health and Human Services, National Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, 1996.

6 Morris JN, Heady JA, Raffle PAB, Roberts CG, Parks JW. Coronary Heart-Disease and Physical Activity of Work. *Lancet* 1953; **262**: 1053–7.

7 U.S. Department of Health and Human Services. Physical activity guidelines for Americans, 2nd edition. Washington, D.C.: U.S. Department of Health and Human Services, 2018.

8 Mendis S, Nordet P, Fernandez-Britto JE, Sternby N. Atherosclerosis in children and young adults: An overview of the World Health Organization and International Society and Federation of Cardiology study on Pathobiological Determinants of Atherosclerosis in Youth study (1985–1995). *Prev Control* 2005; **1**: 3–15.

9 Davis Patricia H., Dawson Jeffrey D., Riley Ward A., Lauer Ronald M. Carotid Intimal-Medial Thickness Is Related to Cardiovascular Risk Factors Measured From Childhood Through Middle Age. *Circulation* 2001; **104**: 2815–9.

10 Raitakari OT, Juonala M, Kähönen M, *et al.* Cardiovascular Risk Factors in Childhood and Carotid Artery Intima-Media Thickness in Adulthood: The Cardiovascular Risk in Young Finns Study. *JAMA* 2003; **290**: 2277–83.

11 Koskinen JS, Kytö V, Juonala M, *et al.* Childhood risk factors and carotid atherosclerotic plaque in adulthood: The Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 2020; **293**: 18–25.

12 Juhola J, Magnussen CG, Viikari JSA, *et al.* Tracking of Serum Lipid Levels, Blood Pressure, and Body Mass Index from Childhood to Adulthood: The Cardiovascular Risk in Young Finns Study. *J Pediatr* 2011; **159**: 584–90.

13 Gillman MW. Primordial Prevention of Cardiovascular Disease. *Circulation* 2015; **131**: 599–601.

14 Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep* 1985; **100**: 126–31.

15 Strath Scott J., Kaminsky Leonard A., Ainsworth Barbara E., *et al.* Guide to the Assessment of Physical Activity: Clinical and Research Applications. *Circulation* 2013; **128**: 2259–79.

16 Leonard WR. Laboratory and field methods for measuring human energy expenditure. *Am J Hum Biol* 2012; **24**: 372–84.

17 Haugen HA, Chan L-N, Li F. Indirect Calorimetry: A Practical Guide for Clinicians. *Nutr Clin Pract* 2007; **22**: 377–88.

18 Westerterp KR. Assessment of physical activity: a critical appraisal. *Eur J Appl Physiol* 2009; **105**: 823–8.

19 Sirard JR, Pate RR. Physical Activity Assessment in Children and Adolescents. *Sports Med* 2001; **31**: 439–54.

20 McKenzie TL, van der Mars H. Top 10 Research Questions Related to Assessing Physical Activity and Its Contexts Using Systematic Observation. *Res Q Exerc Sport* 2015; **86**: 13–29.

21 Warren JM, Ekelund U, Besson H, Mezzani A, Geladas N, Vanhees L. Assessment of physical activity – a review of methodologies with reference to epidemiological research: a report of the exercise physiology section of the European Association of Cardiovascular Prevention and Rehabilitation. *Eur J Cardiovasc Prev Rehabil* 2010; **17**: 127–39.

22 Shephard RJ. Limits to the measurement of habitual physical activity by questionnaires. *Br J Sports Med* 2003; **37**: 197–206; discussion 206.

23 Leonard WR. Measuring human energy expenditure: What have we learned from the flex-heart rate method? *Am J Hum Biol* 2003; **15**: 479–89.

24 Crouter SE, Schneider PL, Karabulut M, Bassett DRJr. Validity of 10 Electronic Pedometers for Measuring Steps, Distance, and Energy Cost. *Med Sci Sports Exerc* 2003; **35**: 1455–60.

25 Eston RG, Rowlands AV, Ingledew DK. Validity of heart rate, pedometry, and accelerometry for predicting the energy cost of children's activities. *J Appl Physiol* 1998; **84**: 362–71.

26 Ainsworth B, Cahalin L, Buman M, Ross R. The Current State of Physical Activity Assessment Tools. *Prog Cardiovasc Dis* 2015; **57**: 387–95.

27 Lee I-M, Shiroma EJ. Using accelerometers to measure physical activity in large-scale epidemiological studies: issues and challenges. *Br J Sports Med* 2014; **48**: 197.

28 Welk GJ, McClain JJ, Ainsworth BE. Protocols for Evaluating Equivalency of Accelerometry-Based Activity Monitors. *Med Sci Sports Exerc* 2012; **44**: S39-49.

29 Cooper AR, Goodman A, Page AS, *et al.* Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). *Int J Behav Nutr Phys Act* 2015; **12**: 113.

30 Resaland GK, Moe VF, Aadland E, *et al.* Active Smarter Kids (ASK): Rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on children's academic performance and risk factors for non-communicable diseases. *BMC Public Health* 2015; **15**: 709.

31 Trost SG, Ward DS, Moorehead SM, Watson PD, Riner W, Burke JR. Validity of the computer science and applications (CSA) activity monitor in children. *Med Sci Sports Exerc* 1998; **30**: 629–33.

32 Ekelund U, Sjöström M, Yngve A, *et al.* Physical activity assessed by activity monitor and doubly labeled water in children. *Med Sci Sports Exerc* 2001; **33**: 275–81.

33 Aadland E, Ylvisåker E. Reliability of the Actigraph GT3X+ Accelerometer in Adults under Free-Living Conditions. *PLOS ONE* 2015; **10**: e0134606.

34 Barreira TV, Schuna JM, Tudor-Locke C, *et al.* Reliability of accelerometer-determined physical activity and sedentary behavior in school-aged children: a 12-country study. *Int J Obes Suppl* 2015; **5**: S29–35.

35 Sedentary Behaviour Research Network. Letter to the Editor: Standardized use of the terms "sedentary" and "sedentary behaviours". *Appl Physiol Nutr Metab* 2012; **37**: 540–2.

36 Tremblay MS, Aubert S, Barnes JD, *et al.* Sedentary Behavior Research Network (SBRN) – Terminology Consensus Project process and outcome. *Int J Behav Nutr Phys Act* 2017; **14**: 75.

37 Ekelund U, Steene-Johannessen J, Brown WJ, *et al.* Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *The Lancet* 2016; **388**: 1302–10.

38 Atkin AJ, Gorely T, Clemes SA, *et al.* Methods of Measurement in epidemiology: Sedentary Behaviour. *Int J Epidemiol* 2012; **41**: 1460–71.

39 Ryan CG, Grant PM, Tigbe WW, Granat MH. The validity and reliability of a novel activity monitor as a measure of walking. *Br J Sports Med* 2006; **40**: 779.

40 Kozey-Keadle S, Libertine A, Lyden K, Staudenmayer J, Freedson PS. Validation of Wearable Monitors for Assessing Sedentary Behavior: *Med Sci Sports Exerc* 2011; **43**: 1561–7.

41 Ross R, Blair SN, Arena R, *et al.* Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation* 2016; **134**: e653–99.

42 Shephard RJ, Allen C, Benade AJ, *et al.* The maximum oxygen intake. An international reference standard of cardiorespiratory fitness. *Bull World Health Organ* 1968; **38**: 757–64.

43 Barker AR, Williams CA, Jones AM, Armstrong N. Establishing maximal oxygen uptake in young people during a ramp cycle test to exhaustion. *Br J Sports Med* 2011; **45**: 498.

44 Armstrong N, Kirby BJ, McManus AM, Welsman JR. Aerobic fitness of prepubescent children. *Ann Hum Biol* 1995; **22**: 427–41.

45 Armstrong N, Welsman J. Development of peak oxygen uptake from 11–16 years determined using both treadmill and cycle ergometry. *Eur J Appl Physiol* 2019; **119**: 801–12.

46 Rowland TW. Does peak VO2 reflect VO2max in children?: evidence from supramaximal testing. *Med Sci Sports Exerc* 1993; **25**: 689–93.

47 Castro-Piñero J, Artero EG, España-Romero V, *et al.* Criterion-related validity of field-based fitness tests in youth: a systematic review. *Br J Sports Med* 2010; **44**: 934.

48 Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. *J Sports Med Phys Fitness* 2008; **48**: 434–7.

49 Armstrong N, Welsman J. Youth cardiorespiratory fitness: evidence, myths and misconceptions. *Bull World Health Organ* 2019; **97**: 777–82.

50 Tomkinson GR, Lang JJ, Léger LA, *et al.* Response to criticisms of the 20 m shuttle run test: deflections, distortions and distractions. *Br J Sports Med* 2019; **53**: 1200.

51 Mayorga-Vega D, Aguilar-Soto P, Viciana J. Criterion-Related Validity of the 20-M Shuttle Run Test for Estimating Cardiorespiratory Fitness: A Meta-Analysis. *J Sports Sci Med* 2015; **14**: 536–47.

52 Aadland E, Terum T, Mamen A, Andersen LB, Resaland GK. The Andersen Aerobic Fitness Test: Reliability and Validity in 10-Year-Old Children. *PLOS ONE* 2014; **9**: e110492.

53 Mach F, Baigent C, Catapano AL, *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* 2019; **41**: 111–88.

54 Balling M, Langsted A, Afzal S, Varbo A, Davey Smith G, Nordestgaard BG. A third of nonfasting plasma cholesterol is in remnant lipoproteins: Lipoprotein subclass profiling in 9293 individuals. *Atherosclerosis* 2019; **286**: 97–104.

55 Sandesara PB, Virani SS, Fazio S, Shapiro MD. The Forgotten Lipids: Triglycerides, Remnant Cholesterol, and Atherosclerotic Cardiovascular Disease Risk. *Endocr Rev* 2018; **40**: 537–57.

56 Nakajima K, Nakano T, Tokita Y, *et al.* Postprandial lipoprotein metabolism: VLDL vs chylomicrons. *Clin Chim Acta* 2011; **412**: 1306–18.

57 Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *The Lancet* 2014; **384**: 626–35.

58 Harahsheh A, Clauss S, Mietus-Snyder M. Beyond the Standard Lipid Profile: What is Known about Apolipoproteins, Lp(a), and Lipoprotein Particle Distributions in Children. *Curr Cardiovasc Risk Rep* 2014; **8**: 1–11.

59 Ference BA, Ginsberg HN, Graham I, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017; **38**: 2459–72.

60 Shapiro MD, Minnier J, Tavori H, *et al.* Relationship Between Low-Density Lipoprotein Cholesterol and Lipoprotein(a) Lowering in Response to PCSK9 Inhibition With Evolocumab. *J Am Heart Assoc* 2019; **8**: e010932.

61 März W, Kleber ME, Scharnagl H, *et al.* HDL cholesterol: reappraisal of its clinical relevance. *Clin Res Cardiol* 2017; **106**: 663–75.

62 Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med* 2011; **17**: 594–603.

63 Reiner Ž, Catapano AL, De Backer G, *et al.* ESC/EAS Guidelines for the management of dyslipidaemias: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J* 2011; **32**: 1769–818.

64 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.

65 Nordestgaard BG. A Test in Context: Lipid Profile, Fasting Versus Nonfasting. *J Am Coll Cardiol* 2017; **70**: 1637–46.

66 Borén J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidol* 2016; **27**: 473–483.

67 Rankin NJ, Preiss D, Welsh P, *et al.* The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective. *Atherosclerosis* 2014; **237**: 287–300.

68 Bictash M, Ebbels TM, Chan Q, *et al.* Opening up the 'Black Box': Metabolic phenotyping and metabolome-wide association studies in epidemiology. *J Clin Epidemiol* 2010; **63**: 970–9.

69 Tzoulaki I, Ebbels TMD, Valdes A, Elliott P, Ioannidis JPA. Design and Analysis of Metabolomics Studies in Epidemiologic Research: A Primer on -Omic Technologies. *Am J Epidemiol* 2014; **180**: 129–39.

70 Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *Am J Epidemiol* 2017; **186**: 1084–96.

71 Mundra PA, Shaw JE, Meikle PJ. Lipidomic analyses in epidemiology. *Int J Epidemiol* 2016; **45**: 1329–38.

72 Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ Cardiovasc Genet* 2015; **8**: 192–206.

73 Mallol R, Rodriguez MA, Brezmes J, Masana L, Correig X. Human serum/plasma lipoprotein analysis by NMR: Application to the study of diabetic dyslipidemia. *Prog Nucl Magn Reson Spectrosc* 2013; **70**: 1–24.

74 Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015; **161**: 161–72.

75 Borén J, Chapman MJ, Krauss RM, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2020; **41**: 2313–30.

76 Richardson TG, Sanderson E, Palmer TM, *et al.* Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. *PLOS Med* 2020; **17**: e1003062.

77 Ala-Korpela M. The culprit is the carrier, not the loads: cholesterol, triglycerides and apolipoprotein B in atherosclerosis and coronary heart disease. *Int J Epidemiol* 2019; **48**: 1389–92.

78 Ference BA, Kastelein JJP, Ray KK, *et al.* Association of Triglyceride-Lowering LPL Variants and LDL-C–Lowering LDLR Variants With Risk of Coronary Heart Disease. *JAMA* 2019; **321**: 364–73.

79 Nordestgaard BG. Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. *Circ Res* 2016; **118**: 547–63.

80 Chait A, Ginsberg HN, Vaisar T, Heinecke JW, Goldberg IJ, Bornfeldt KE. Remnants of the Triglyceride-Rich Lipoproteins, Diabetes, and Cardiovascular Disease. *Diabetes* 2020; **69**: 508–16.

81 Lincoff AM, Nicholls SJ, Riesmeyer JS, *et al.* Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. *N Engl J Med* 2017; **376**: 1933–42.

82 Voight BF, Peloso GM, Orho-Melander M, *et al.* Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *The Lancet* 2012; **380**: 572–80.

83 Akinkuolie AO, Paynter NP, Padmanabhan L, Mora S. High-Density Lipoprotein Particle Subclass Heterogeneity and Incident Coronary Heart Disease. *Circ Cardiovasc Qual Outcomes* 2014; **7**: 55–63.

84 Holmes MV, Millwood IY, Kartsonaki C, *et al.* Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *J Am Coll Cardiol* 2018; **71**: 620–32.

85 Ekelund U, Tarp J, Steene-Johannessen J, *et al.* Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: systematic review and harmonised meta-analysis. *BMJ* 2019; **366**: l4570.

86 Kodama S, Saito K, Tanaka S, *et al.* Cardiorespiratory Fitness as a Quantitative Predictor of All-Cause Mortality and Cardiovascular Events in Healthy Men and Women: A Meta-analysis. *JAMA* 2009; **301**: 2024–35.

87 Kraus WE, Powell KE, Haskell WL, *et al.* Physical Activity, All-Cause and Cardiovascular Mortality, and Cardiovascular Disease. *Med Sci Sports Exerc* 2019; **51**: 1270–1281.

88 Clausen JSR, Marott JL, Holtermann A, Gyntelberg F, Jensen MT. Midlife Cardiorespiratory Fitness and the Long-Term Risk of Mortality. *J Am Coll Cardiol* 2018; **72**: 987.

89 Biswas A, Oh PI, Faulkner GE, *et al.* Sedentary Time and Its Association With Risk for Disease Incidence, Mortality, and Hospitalization in Adults. *Ann Intern Med* 2015; **162**: 123–32.

90 Wilmot EG, Edwardson CL, Achana FA, *et al.* Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia* 2012; **55**: 2895–905.

91 Piepoli MF, Hoes AW, Agewall S, *et al.* 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of

Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016; **37**: 2315–81.

92 Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; **23**: 469–80.

93 Grundy Scott M., Cleeman James I., Daniels Stephen R., *et al.* Diagnosis and Management of the Metabolic Syndrome. *Circulation* 2005; **112**: 2735–52.

94 Mottillo S, Filion KB, Genest J, *et al.* The Metabolic Syndrome and Cardiovascular Risk: A Systematic Review and Meta-Analysis. *J Am Coll Cardiol* 2010; **56**: 1113–32.

95 Magge SN, Goodman E, Armstrong SC. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. *Pediatrics* 2017; **140**: e20171603.

96 Goodman Elizabeth, Daniels Stephen R., Meigs James B., Dolan Lawrence M. Instability in the Diagnosis of Metabolic Syndrome in Adolescents. *Circulation* 2007; **115**: 2316–22.

97 Magnussen Costan G., Koskinen Juha, Chen Wei, *et al.* Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus but Is No Better Than Body Mass Index Alone. *Circulation* 2010; **122**: 1604–11.

98 Koskinen Juha, Magnussen Costan G., Sinaiko Alan, *et al.* Childhood Age and Associations Between Childhood Metabolic Syndrome and Adult Risk for Metabolic Syndrome, Type 2 Diabetes Mellitus and Carotid Intima Media Thickness: The International Childhood Cardiovascular Cohort Consortium. *J Am Heart Assoc* 2017; **6**: e005632.

99 Bayer O, Krüger H, Von Kries R, Toschke AM. Factors Associated With Tracking of BMI: A Meta-Regression Analysis on BMI Tracking. *Obesity* 2011; **19**: 1069–76.

100 Simmonds M, Burch J, Llewellyn A, *et al.* The use of measures of obesity in childhood for predicting obesity and the development of obesity-related diseases in adulthood: a systematic review and meta-analysis. *Health Technol Assess* 2015; **19**: 1–336.

101 Srinivasan SR, Myers L, Berenson GS. Predictability of Childhood Adiposity and Insulin for Developing Insulin Resistance Syndrome (Syndrome X) in Young Adulthood. *Diabetes* 2002; **51**: 204.

102 Lloyd LJ, Langley-Evans SC, McMullen S. Childhood obesity and risk of the adult metabolic syndrome: a systematic review. *Int J Obes* 2012; **36**: 1–11.

103 Skrede T, Steene-Johannessen J, Anderssen SA, Resaland GK, Ekelund U. The prospective association between objectively measured sedentary time, moderate-to-vigorous physical activity and cardiometabolic risk factors in youth: a systematic review and meta-analysis. *Obes Rev* 2019; **20**: 55–74.

104 Skrede T, Stavnsbo M, Aadland E, *et al.* Moderate-to-vigorous physical activity, but not sedentary time, predicts changes in cardiometabolic risk factors in 10-y-old children: the Active Smarter Kids Study. *Am J Clin Nutr* 2017; **105**: 1391–8.

105 Kriemler S, Zahner L, Schindler C, *et al.* Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial. *BMJ* 2010; **340**: c785.

106 Meyer U, Schindler C, Zahner L, *et al.* Long-Term Effect of a School-Based Physical Activity Program (KISS) on Fitness and Adiposity in Children: A Cluster-Randomized Controlled Trial. *PLOS ONE* 2014; **9**: e87929.

107 Stavnsbo M, Aadland E, Anderssen SA, *et al.* Effects of the Active Smarter Kids (ASK) physical activity intervention on cardiometabolic risk factors in children: A cluster-randomized controlled trial. *Prev Med* 2020; **130**: 105868.

108 Sun C, Pezic A, Tikellis G, *et al.* Effects of school-based interventions for direct delivery of physical activity on fitness and cardiometabolic markers in children and adolescents: a systematic review of randomized controlled trials. *Obes Rev* 2013; **14**: 818–38.

109 Jones M, Defever E, Letsinger A, Steele J, Mackintosh KA. A mixed-studies systematic review and meta-analysis of school-based interventions to promote physical activity and/or reduce sedentary time in children. *J Sport Health Sci* 2020; **9**: 3–17.

110 Chaput J-P, Saunders TJ, Mathieu M-È, *et al.* Combined associations between moderate to vigorous physical activity and sedentary behaviour with cardiometabolic risk factors in children. *Appl Physiol Nutr Metab* 2013; **38**: 477–83.

111 Ekelund U, Luan J, Sherar LB, *et al.* Moderate to Vigorous Physical Activity and Sedentary Time and Cardiometabolic Risk Factors in Children and Adolescents. *JAMA* 2012; **307**: 704–12.

112 Stamatakis E, Coombs N, Tiling K, *et al.* Sedentary Time in Late Childhood and Cardiometabolic Risk in Adolescence. *Pediatrics* 2015; **135**: e1432.

113 Hjorth MF, Chaput J-P, Damsgaard CT, *et al.* Low Physical Activity Level and Short Sleep Duration Are Associated with an Increased Cardio-Metabolic Risk Profile: A Longitudinal Study in 8-11 Year Old Danish Children. *PLOS ONE* 2014; **9**: e104677.

114 Väistö J, Haapala EA, Viitasalo A, *et al.* Longitudinal associations of physical activity and sedentary time with cardiometabolic risk factors in children. *Scand J Med Sci Sports* 2019; **29**: 113–23.

115 Tarp J, Bugge A, Andersen LB, *et al.* Does adiposity mediate the relationship between physical activity and biological risk factors in youth?: a cross-sectional study from the International Children's Accelerometry Database (ICAD). *Int J Obes* 2018; **42**: 671–8.

116 Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM. Cardiovascular disease risk in healthy children and its association with body mass index: systematic review and meta-analysis. *BMJ* 2012; **345**: e4759.

117 Falaschetti E, Hingorani AD, Jones A, *et al.* Adiposity and cardiovascular risk factors in a large contemporary population of pre-pubertal children. *Eur Heart J* 2010; **31**: 3063–72.

118 Richmond RC, Davey Smith G, Ness AR, den Hoed M, McMahon G, Timpson NJ. Assessing Causality in the Association between Child Adiposity and Physical Activity Levels: A Mendelian Randomization Analysis. *PLoS Med* 2014; **11**: e1001618.

119 Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The Role of Exercise and Physical Activity in Weight Loss and Maintenance. *Prog Cardiovasc Dis* 2014; **56**: 441–7.

120 Brocklebank LA, Falconer CL, Page AS, Perry R, Cooper AR. Accelerometer-measured sedentary time and cardiometabolic biomarkers: A systematic review. *Prev Med* 2015; **76**: 92–102.

121 Hadgraft NT, Winkler E, Climie RE, *et al.* Effects of sedentary behaviour interventions on biomarkers of cardiometabolic risk in adults: systematic review with meta-analyses. *Br J Sports Med* 2020; : bjsports-2019-101154.

122 Carson V, Hunter S, Kuzik N, *et al.* Systematic review of sedentary behaviour and health indicators in school-aged children and youth: an update. *Appl Physiol Nutr Metab* 2016; **41**: S240–65.

123 Cliff DP, Hesketh KD, Vella SA, *et al.* Objectively measured sedentary behaviour and health and development in children and adolescents: systematic review and meta-analysis: Child sedentary behaviour and health. *Obes Rev* 2016; **17**: 330–44.

124 van Ekris E, Altenburg TM, Singh AS, Proper KI, Heymans MW, Chinapaw MJM. An evidence-update on the prospective relationship between childhood sedentary behaviour and biomedical health indicators: a systematic review and meta-analysis. *Obes Rev* 2016; **17**: 833–49.

125 Mekary RA, Willett WC, Hu FB, Ding EL. Isotemporal Substitution Paradigm for Physical Activity Epidemiology and Weight Change. *Am J Epidemiol* 2009; **170**: 519–27.

126 Hansen BH, Anderssen SA, Andersen LB, *et al.* Cross-Sectional Associations of Reallocating Time Between Sedentary and Active Behaviours on Cardiometabolic Risk Factors in Young People: An International Children's Accelerometry Database (ICAD) Analysis. *Sports Med* 2018; **48**: 2401–12.

127 Wijndaele K, White T, Andersen LB, *et al.* Substituting prolonged sedentary time and cardiovascular risk in children and youth: a meta-analysis within the International Children's Accelerometry database (ICAD). *Int J Behav Nutr Phys Act* 2019; **16**: 96.

128 Anderssen SA, Cooper AR, Riddoch C, *et al.* Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 526–31.

129 Zaqout M, Michels N, Bammann K, *et al.* Influence of physical fitness on cardio-metabolic risk factors in European children. The IDEFICS study. *Int J Obes* 2016; **40**: 1119.

130 Ekelund U, Anderssen SA, Froberg K, *et al.* Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. *Diabetologia* 2007; **50**: 1832–40.

131 Lätt E, Mäestu J, Rääsk T, Jürimäe T, Jürimäe J. Cardiovascular fitness, physical activity, and metabolic syndrome risk factors among adolescent estonian boys: A longitudinal study. *Am J Hum Biol* 2016; **28**: 782–8.

132 Mintjens S, Menting MD, Daams JG, van Poppel MNM, Roseboom TJ, Gemke RJBJ. Cardiorespiratory Fitness in Childhood and Adolescence Affects Future Cardiovascular Risk Factors: A Systematic Review of Longitudinal Studies. *Sports Med* 2018; **48**: 2577–605.

133 Fairchild TJ, Klakk H, Heidemann MS, Andersen LB, Wedderkopp N. Exploring the Relationship between Adiposity and Fitness in Young Children. *Med Sci Sports Exerc* 2016; **48**: 1708–14.

134 Zaqout M, Vyncke K, Moreno LA, *et al.* Determinant factors of physical fitness in European children. *Int J Public Health* 2016; **61**: 573–82.

135 Church TS, Earnest CP, Skinner JS, Blair SN. Effects of Different Doses of Physical Activity on Cardiorespiratory Fitness Among Sedentary, Overweight or Obese Postmenopausal Women With Elevated Blood PressureA Randomized Controlled Trial. *JAMA* 2007; **297**: 2081–91.

136 Bouchard C, An P, Rice T, *et al.* Familial aggregation of VO2max response to exercise training: results from the HERITAGE Family Study. *J Appl Physiol* 1999; **87**: 1003–8.

137 Bouchard C, Blair SN, Katzmarzyk PT. Less Sitting, More Physical Activity, or Higher Fitness? *Mayo Clin Proc* 2015; **90**: 1533–40.

138 Chomistek AK, Chasman DI, Cook NR, Rimm EB, Lee I-M. Physical Activity, Genes for Physical Fitness, and Risk of Coronary Heart Disease. *Med Sci Sports Exerc* 2013; **45**: 691–7.

139 Skrede T, Aadland E, Andersen LB, *et al.* Does cardiorespiratory fitness moderate the prospective association between physical activity and cardiometabolic risk factors in children? *Int J Obes* 2018; **42**: 1029–38.

140 Kujala UM, Vaara JP, Kainulainen H, Vasankari T, Vaara E, Kyröläinen H. Associations of Aerobic Fitness and Maximal Muscular Strength With Metabolites in Young Men. *JAMA Netw Open* 2019; **2**: e198265–e198265.

141 Kujala UM, Makinen V-P, Heinonen I, *et al.* Long-term Leisure-time Physical Activity and Serum Metabolome. *Circulation* 2013; **127**: 340–8.

142 Resaland GK, Aadland E, Moe VF, *et al.* Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. *Prev Med* 2016; **91**: 322–8.

143 World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* 2013; **310**: 2191–4.

144 Esliger DW, Copeland JL, Barnes JD, Tremblay MS. Standardizing and Optimizing the Use of Accelerometer Data for Free-Living Physical Activity Monitoring. *J Phys Act Health* 2005; **2**: 366–83.

145 Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. *J Sports Sci* 2008; **26**: 1557–65.

146 Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of Accelerometer Cut Points for Predicting Activity Intensity in Youth: *Med Sci Sports Exerc* 2011; **43**: 1360–8.

147 Okazaki M. Identification of Unique Lipoprotein Subclasses for Visceral Obesity by Component Analysis of Cholesterol Profile in High-Performance Liquid Chromatography. *Arterioscler Thromb Vasc Biol* 2005; **25**: 578–84.

148 Kvalheim OM, Arneberg R, Grung B, Rajalahti T. Determination of optimum number of components in partial least squares regression from distributions of the root-mean-squared error obtained by Monte Carlo resampling. *J Chemom* 2018; **32**: e2993.

149 Lin C, Rajalahti T, Mjøs SA, Kvalheim OM. Predictive associations between serum fatty acids and lipoproteins in healthy non-obese Norwegians: implications for cardiovascular health. *Metabolomics* 2015; **12**: 6.

150 Okazaki M, Yamashita S. Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using “Spherical Particle Model”. *J Oleo Sci* 2016; **65**: 265–82.

151 Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–94.

152 Carel J-C, Léger J. Precocious Puberty. *N Engl J Med* 2008; **358**: 12.

153 R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020 https://www.R-project.org/.

154 Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J R Stat Soc Ser B Methodol* 1995; **57**: 289–300.

155 Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008; **32**: 361–9.

156 Ferreira DLS, Williams DM, Kangas AJ, *et al.* Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. *PLOS Med* 2017; **14**: e1002376.

157 Spiller SA, Fitzsimons GJ, Lynch JG, Mcclelland GH. Spotlights, Floodlights, and the Magic Number Zero: Simple Effects Tests in Moderated Regression. *J Mark Res* 2013; **50**: 277–88.

158 Bell JA, Hamer M, Richmond RC, Timpson NJ, Carslake D, Smith GD. Associations of device-measured physical activity across adolescence with metabolic traits: Prospective cohort study. *PLOS Med* 2018; **15**: e1002649.

159 Cliff DP, Okely AD, Burrows TL, *et al.* Objectively measured sedentary behavior, physical activity, and plasma lipids in overweight and obese children. *Obesity* 2013; **21**: 382–5.

160 Kelley GA, Kelley KS. Aerobic exercise and lipids and lipoproteins in children and adolescents: A meta-analysis of randomized controlled trials. *Atherosclerosis* 2007; **191**: 447–53.

161 LeBlanc AG, Janssen I. Dose-response relationship between physical activity and dyslipidemia in youth. *Can J Cardiol* 2010; **26**: e201–5.

162 Blauw LL, Noordam R, Soidinsalo S, *et al.* Mendelian randomization reveals unexpected effects of CETP on the lipoprotein profile. *Eur J Hum Genet* 2019; **27**: 422–31.

163 Seip R L, Moulin P, Cocke T, *et al.* Exercise training decreases plasma cholesteryl ester transfer protein. *Arterioscler Thromb J Vasc Biol* 1993; **13**: 1359–67.

164 Escalante Y, Saavedra JM, García-Hermoso A, Domínguez AM. Improvement of the lipid profile with exercise in obese children: A systematic review. *Prev Med* 2012; **54**: 293–301.

165 Carson V, Ridgers ND, Howard BJ, *et al.* Light-Intensity Physical Activity and Cardiometabolic Biomarkers in US Adolescents. *PLOS ONE* 2013; **8**: e71417.

166 Carson V, Tremblay MS, Chaput J-P, McGregor D, Chastin S. Compositional analyses of the associations between sedentary time, different intensities of physical activity, and cardiometabolic biomarkers among children and youth from the United States. *PLOS ONE* 2019; **14**: e0220009.

167 Hamer M, Stamatakis E, Steptoe A. Effects of Substituting Sedentary Time with Physical Activity on Metabolic Risk. *Med Sci Sports Exerc* 2014; **46**: 1946–50.

168 Resaland GK, Rajalahti T, Aadland E, Kvalheim OM. Strong association between cardiorespiratory fitness and serum lipoprotein subclass pattern in prepubertal healthy children. *Scand J Med Sci Sports* 2018; **28**: 220–7.

169 Herd SL, Hardman AE, Boobis LH, Cairns CJ. The effect of 13 weeks of running training followed by 9 d of detraining on postprandial lipaemia. *Br J Nutr* 1998; **80**: 57–66.

170 Goran M, Fields DA, Hunter GR, Herd SL, Weinsier RL. Total body fat does not influence maximal aerobic capacity. *Int J Obes Relat Metab Disord J Int Assoc Study Obes* 2000; **24**: 841–8.

171 Würtz P, Wang Q, Kangas AJ, *et al.* Metabolic Signatures of Adiposity in Young Adults: Mendelian Randomization Analysis and Effects of Weight Change. *PLoS Med* 2014; **11**: e1001765.

172 Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia* 2015; **58**: 886–99.

173 Ginsberg HN, Zhang Y-L, Hernandez-Ono A. Regulation of Plasma Triglycerides in Insulin Resistance and Diabetes. *Arch Med Res* 2005; **36**: 232–40.

174 Bays HE, Toth PP, Kris-Etherton PM, *et al.* Obesity, adiposity, and dyslipidemia: A consensus statement from the National Lipid Association. *J Clin Lipidol* 2013; **7**: 304–83.

175 Sniderman AD, Couture P, Martin SS, *et al.* Hypertriglyceridemia and cardiovascular risk: a cautionary note about metabolic confounding. *J Lipid Res* 2018; **59**: 1266–75.

176 Ference BA, Kastelein JJP, Ginsberg HN, *et al.* Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk. *JAMA* 2017; **318**: 947–56.

177 Albers JJ, Cabana VG, Hazzard WR. Immunoassay of human plasma apolipoprotein B. *Metabolism* 1975; **24**: 1339–51.

178 Nordestgaard BG, Benn M, Schnohr P, Tybjærg-Hansen A. Nonfasting Triglycerides and Risk of Myocardial Infarction, Ischemic Heart Disease, and Death in Men and Women. *JAMA* 2007; **298**: 299–308.

179 Ference BA, Graham I, Tokgozoglu L, Catapano AL. Impact of Lipids on Cardiovascular Health: JACC Health Promotion Series. *J Am Coll Cardiol* 2018; **72**: 1141–56.

180 Horowitz JF, Klein S. Lipid metabolism during endurance exercise. *Am J Clin Nutr* 2000; **72**: 558S-563S.

181 Sondergaard E, Rahbek I, Sørensen LP, *et al.* Effects of exercise on VLDL-triglyceride oxidation and turnover. *Am J Physiol-Endocrinol Metab* 2011; **300**: E939–44.

182 Magkos F, Wright DC, Patterson BW, Mohammed BS, Mittendorfer B. Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. *Am J Physiol-Endocrinol Metab* 2006; **290**: E355–62.

183 Herd SL, Lawrence JEM, Malkova D, Murphy MH, Mastana S, Hardman AE. Postprandial lipemia in young men and women of contrasting training status. *J Appl Physiol* 2000; **89**: 2049–56.

184 Holmes MV, Asselbergs FW, Palmer TM, *et al.* Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J* 2014; **36**: 539–50.

185 Ruiz-Ramie JJ, Barber JL, Sarzynski MA. Effects of exercise on HDL functionality. *Curr Opin Lipidol* 2019; **30**: 16–23.

186 Allard-Ratick MP, Kindya BR, Khambhati J, *et al.* HDL: Fact, fiction, or function? HDL cholesterol and cardiovascular risk. *Eur J Prev Cardiol* 2019; : 2047487319848214.

187 Jomard A, Osto E. High Density Lipoproteins: Metabolism, Function, and Therapeutic Potential. *Front Cardiovasc Med* 2020; **7**: 39.

188 Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1·6 million participants. *Lancet Child Adolesc Health* 2020; **4**: 23–35.

189 WHO Regional Office for Europe. WHO European Childhood Obesity Surveillance Initiative: overweight and obesity among 6–9-year-old children. Report of the third round of data collection 2012–2013. Copenhagen: World Health Organization, 2018.

190 Kwon S, Andersen LB, Grøntved A, *et al.* A closer look at the relationship among accelerometer-based physical activity metrics: ICAD pooled data. *Int J Behav Nutr Phys Act* 2019; **16**: 40.

191 Mattocks C, Leary S, Ness A, *et al.* Intraindividual Variation of Objectively Measured Physical Activity in Children. *Med Sci Sports Exerc* 2007; **39**: 622–9.

192 Baquet G, Stratton G, Van Praagh E, Berthoin S. Improving physical activity assessment in prepubertal children with high-frequency accelerometry monitoring: A methodological issue. *Prev Med* 2007; **44**: 143–7.

193 McClain, James J. JJ, Abraham TL, Brusseau TAJr, Tudor-Locke C. Epoch Length and Accelerometer Outputs in Children: Comparison to Direct Observation. *Med Sci Sports Exerc* 2008; **40**: 2080–7.

194 Herman Hansen B, Børtnes I, Hildebrand M, Holme I, Kolle E, Anderssen SA. Validity of the ActiGraph GT1M during walking and cycling. *J Sports Sci* 2014; **32**: 510–6.

195 Mattsson E, Larsson UE, Rössner S. Is walking for exercise too exhausting for obese women? *Int J Obes* 1997; **21**: 380–6.

196 Aadland E, Kvalheim OM, Rajalahti T, Skrede T, Resaland GK. Aerobic fitness and metabolic health in children: A clinical validation of directly measured maximal oxygen consumption versus performance measures as markers of health. *Prev Med Rep* 2017; **7**: 74–6.

197 Rich C, Geraci M, Griffiths L, Sera F, Dezateux C, Cortina-Borja M. Quality Control Methods in Accelerometer Data Processing: Defining Minimum Wear Time. *PLOS ONE* 2013; **8**: e67206.

198 Janssen X, Basterfield L, Parkinson KN, *et al.* Objective measurement of sedentary behavior: impact of non-wear time rules on changes in sedentary time. *BMC Public Health* 2015; **15**: 504.

199 Manousaki D, Barnett TA, Mathieu M-E, *et al.* Tune out and turn in: the influence of television viewing and sleep on lipid profiles in children. *Int J Obes* 2020; **44**: 1173–84.

200 Bender R, Lange S. Adjusting for multiple testing—when and how? *J Clin Epidemiol* 2001; **54**: 343–9.

201 Wasserstein RL, Lazar NA. The ASA's Statement on *p* -Values: Context, Process, and Purpose. *Am Stat* 2016; **70**: 129–33.

202 Greenland S, Senn SJ, Rothman KJ, *et al.* Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. *Eur J Epidemiol* 2016; **31**: 337–50.

203 Sacks FM, Jensen MK. From High-Density Lipoprotein Cholesterol to Measurements of Function: Prospects for the Development of Tests for High-Density Lipoprotein Functionality in Cardiovascular Disease. *Arterioscler Thromb Vasc Biol* 2018; **38**: 487–99.

204 Khan SS, Ning H, Wilkins JT, *et al.* Association of Body Mass Index With Lifetime Risk of Cardiovascular Disease and Compression of Morbidity. *JAMA Cardiol* 2018; **3**: 280–7.

205 Di Angelantonio E, Bhupathiraju SN, Wormser D, *et al.* Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *The Lancet* 2016; **388**: 776–86.

206 Tzoulaki I, Castagné R, Boulangé CL, *et al.* Serum metabolic signatures of coronary and carotid atherosclerosis and subsequent cardiovascular disease. *Eur Heart J* 2019; **40**: 2883–96.

207 Würtz P, Wang Q, Soininen P, *et al.* Metabolomic Profiling of Statin Use and Genetic Inhibition of HMG-CoA Reductase. *J Am Coll Cardiol* 2016; **67**: 1200–10.

208 Deelen J, Kettunen J, Fischer K, *et al.* A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nat Commun* 2019; **10**: 3346.

209 Mackey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S. High-Density Lipoprotein Cholesterol and Particle Concentrations, Carotid Atherosclerosis, and Coronary Events: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2012; **60**: 508–16.

210 Cromwell WC, Otvos JD, Keyes MJ, *et al.* LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study—Implications for LDL management. *J Clin Lipidol* 2007; **1**: 583–92.

211 Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein Particle Profiles by Nuclear Magnetic Resonance Compared With Standard Lipids and Apolipoproteins in Predicting Incident Cardiovascular Disease in Women. *Circulation* 2009; **119**: 931–9.

212 Ohukainen P, Kuusisto S, Kettunen J, *et al.* Data-driven multivariate population subgrouping via lipoprotein phenotypes versus apolipoprotein B in the risk assessment of coronary heart disease. *Atherosclerosis* 2020; **294**: 10–5.

213 Cole TG, Contois JH, Csako G, *et al.* Association of Apolipoprotein B and Nuclear Magnetic Resonance Spectroscopy–Derived LDL Particle Number with Outcomes in 25 Clinical Studies: Assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices. *Clin Chem* 2013; **59**: 752–70.

214 Horvath AR, Lord SJ, StJohn A, *et al.* From biomarkers to medical tests: The changing landscape of test evaluation. *Clin Chim Acta* 2014; **427**: 49–57.

Supplementary material

Supplementary table 1. Associations of 30 minutes' MVPA and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.0263 (–0.0417, –0.0109)	–0.022 (–0.0343, –0.00976)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.127 (–0.204, –0.0492)	–0.122 (–0.17, –0.0729)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.628 (–0.942, –0.314)	–0.54 (–0.788, –0.293)
Concentration of VLDL L3 particles (nmol·L^{-1})	–2.05 (–2.87, –1.22)	–1.35 (–2.15, –0.556)
Concentration of VLDL M particles (nmol·L^{-1})	–2.11 (–3.17, –1.05)	–1.4 (–2.45, –0.358)
Concentration of VLDL S particles (nmol·L^{-1})	0.242 (–0.675, 1.16)	0.553 (–0.373, 1.48)
Concentration of LDL L particles (nmol·L^{-1})	1.5 (–2.21, 5.21)	2.95 (–0.79, 6.69)
Concentration of LDL M particles (nmol·L^{-1})	–3.6 (–11.9, 4.72)	1.54 (–6.7, 9.79)
Concentration of LDL S particles (nmol·L^{-1})	–4.61 (–8.69, –0.541)	–1.49 (–5.47, 2.49)
Concentration of LDL VS particles (nmol·L^{-1})	–2.01 (–4, –0.0176)	–0.649 (–2.61, 1.32)
Concentration of HDL VL particles (nmol·L^{-1})	8.47 (1.22, 15.7)	2.17 (–4.83, 9.17)
Concentration of HDL L particles (nmol·L^{-1})	93.5 (35.4, 152)	37.2 (–18.1, 92.5)
Concentration of HDL M particles (nmol·L^{-1})	92.8 (46.1, 140)	65 (18.6, 111)
Concentration of HDL S particles (nmol·L^{-1})	–13 (–55.8, 29.9)	13.7 (–28.8, 56.2)
Concentration of HDL VS particles (nmol·L^{-1})	–11.8 (–38.2, 14.7)	–1.13 (–27.7, 25.5)
Total cholesterol concentration (mmol·L^{-1})	–0.00658 (–0.0623, 0.0492)	0.0157 (–0.0405, 0.0718)
Cholesterol in CM particles (mmol·L^{-1})	–0.00128 (–0.00197, –0.000598)	–0.00126 (–0.00187, –0.000656)
Cholesterol in VLDL particles (mmol·L^{-1})	–0.0333 (–0.0537, –0.0129)	–0.0173 (–0.0372, 0.00262)
Cholesterol in LDL particles (mmol·L^{-1})	–0.0122 (–0.0531, 0.0288)	0.011 (–0.0297, 0.0518)
Cholesterol in HDL particles (mmol·L^{-1})	0.0383 (0.018, 0.0586)	0.0209 (0.00129, 0.0405)
Total triglycerides concentration (mmol·L^{-1})	–0.0469 (–0.0785, –0.0153)	–0.0406 (–0.0653, –0.0159)
Triglycerides in CM particles (mmol·L^{-1})	–0.00248 (–0.00406, –0.000894)	–0.00242 (–0.00368, –0.00116)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.0416 (–0.072, –0.0112)	–0.0368 (–0.0585, –0.0151)
Triglycerides in LDL particles (mmol·L^{-1})	–0.00255 (–0.00531, 0.000211)	–0.000811 (–0.00355, 0.00192)
Triglycerides in HDL particles (mmol·L^{-1})	–0.00313 (–0.00613, –0.000135)	–0.00224 (–0.00528, 0.000788)
Mean diameter for VLDL particles (nm)	–0.468 (–0.661, –0.275)	–0.415 (–0.609, –0.22)

Mean diameter for LDL particles (nm)	0.0246 (0.014, 0.0352)	0.019 (0.00838, 0.0295)
Mean diameter for HDL particles (nm)	0.0276 (0.0108, 0.0444)	0.0105 (–0.00533, 0.0264)

Each regression analysis was adjusted for monitor wear time, parents' education, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Supplementary table 2. Associations of 30 minutes' LPA and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.00251 (–0.0144, 0.00941)	–0.000866 (–0.01, 0.00828)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.00093 (–0.0537, 0.0519)	–0.0165 (–0.0654, 0.0324)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.0989 (–0.335, 0.137)	–0.098 (–0.31, 0.114)
Concentration of VLDL L3 particles (nmol·L^{-1})	–0.154 (–0.78, 0.473)	–0.123 (–0.716, 0.469)
Concentration of VLDL M particles (nmol·L^{-1})	–0.226 (–1.03, 0.577)	–0.195 (–0.97, 0.58)
Concentration of VLDL S particles (nmol·L^{-1})	0.286 (–0.402, 0.974)	0.298 (–0.386, 0.982)
Concentration of LDL L particles (nmol·L^{-1})	2.6 (–0.179, 5.39)	2.66 (–0.0992, 5.42)
Concentration of LDL M particles (nmol·L^{-1})	6.41 (0.176, 12.6)	6.61 (0.543, 12.7)
Concentration of LDL S particles (nmol·L^{-1})	2.61 (–0.448, 5.67)	2.74 (–0.19, 5.67)
Concentration of LDL VS particles (nmol·L^{-1})	1.11 (–0.387, 2.61)	1.17 (–0.281, 2.61)
Concentration of HDL VL particles (nmol·L^{-1})	–1.7 (–7.16, 3.75)	–1.96 (–7.13, 3.2)
Concentration of HDL L particles (nmol·L^{-1})	–8.25 (–52.1, 35.6)	–10.6 (–51.4, 30.3)
Concentration of HDL M particles (nmol·L^{-1})	–1.48 (–36.8, 33.8)	–2.71 (–37.1, 31.7)
Concentration of HDL S particles (nmol·L^{-1})	22.1 (–10, 54.2)	23.2 (–8.16, 54.5)
Concentration of HDL VS particles (nmol·L^{-1})	13.7 (–6.15, 33.5)	14.1 (–5.5, 33.7)
Total cholesterol concentration (mmol·L^{-1})	0.0322 (–0.00958, 0.074)	0.0331 (–0.00828, 0.0745)
Cholesterol in CM particles (mmol·L^{-1})	–0.000111 (–0.000701, 0.000478)	–0.000172 (–0.000684, 0.000339)
Cholesterol in VLDL particles (mmol·L^{-1})	0.000896 (–0.0145, 0.0163)	0.00157 (–0.0132, 0.0163)
Cholesterol in LDL particles (mmol·L^{-1})	0.0316 (0.000958, 0.0623)	0.0325 (0.00254, 0.0626)
Cholesterol in HDL particles (mmol·L^{-1})	0.00199 (–0.0133, 0.0173)	0.00126 (–0.0132, 0.0158)
Total triglycerides concentration (mmol·L^{-1})	–0.00279 (–0.0241, 0.0185)	–0.00351 (–0.0244, 0.0174)
Triglycerides in CM particles (mmol·L^{-1})	–0.0000225 (–0.00118, 0.00113)	–0.0005 (–0.00157, 0.000566)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.00468 (–0.0233, 0.014)	–0.0035 (–0.0185, 0.0115)
Triglycerides in LDL particles (mmol·L^{-1})	–0.000467 (–0.00254, 0.00161)	–0.000395 (–0.00241, 0.00162)
Triglycerides in HDL particles (mmol·L^{-1})	–0.000883 (–0.00314, 0.00137)	–0.000844 (–0.00308, 0.0014)
Mean diameter for VLDL particles (nm)	–0.0861 (–0.232, 0.06)	–0.0665 (–0.195, 0.0622)

Mean diameter for LDL particles (nm)	0.00247 (–0.00558, 0.0105)	0.00222 (–0.00565, 0.0101)
Mean diameter for HDL particles (nm)	–0.00456 (–0.0172, 0.00812)	–0.00527 (–0.017, 0.00646)

Each regression analysis was adjusted for monitor wear time, parents' education, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Supplementary table 3. Associations of 30 minutes' sedentary time and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	0.00927 (0.00133, 0.0172)	0.00767 (0.0012, 0.0141)
Concentration of VLDL L1 particles (nmol·L^{-1})	0.042 (0.00633, 0.0776)	0.0429 (0.00847, 0.0773)
Concentration of VLDL L2 particles (nmol·L^{-1})	0.247 (0.0965, 0.397)	0.173 (0.0284, 0.318)
Concentration of VLDL L3 particles (nmol·L^{-1})	0.627 (0.196, 1.06)	0.416 (0.00505, 0.826)
Concentration of VLDL M particles (nmol·L^{-1})	0.679 (0.126, 1.23)	0.464 (–0.0738, 1)
Concentration of VLDL S particles (nmol·L^{-1})	–0.2 (–0.675, 0.276)	–0.288 (–0.763, 0.186)
Concentration of LDL L particles (nmol·L^{-1})	–1.64 (–3.56, 0.282)	–2.06 (–3.97, –0.143)
Concentration of LDL M particles (nmol·L^{-1})	–2.08 (–6.39, 2.23)	–3.6 (–7.83, 0.617)
Concentration of LDL S particles (nmol·L^{-1})	0.00152 (–2.12, 2.12)	–0.935 (–2.97, 1.1)
Concentration of LDL VS particles (nmol·L^{-1})	0.0136 (–1.02, 1.05)	–0.395 (–1.4, 0.612)
Concentration of HDL VL particles (nmol·L^{-1})	–1.49 (–5.26, 2.28)	0.373 (–3.21, 3.96)
Concentration of HDL L particles (nmol·L^{-1})	–21.4 (–51.7, 8.82)	–4.68 (–33.1, 23.7)
Concentration of HDL M particles (nmol·L^{-1})	–24.2 (–48.6, 0.172)	–15.6 (–39.4, 8.25)
Concentration of HDL S particles (nmol·L^{-1})	–6.7 (–28.9, 15.5)	–14.5 (–36.3, 7.24)
Concentration of HDL VS particles (nmol·L^{-1})	–3.18 (–16.9, 10.5)	–6.37 (–20, 7.27)
Total cholesterol concentration (mmol·L^{-1})	–0.0135 (–0.0424, 0.0154)	–0.0201 (–0.0488, 0.00865)
Cholesterol in CM particles (mmol·L^{-1})	0.000486 (0.000132, 0.00084)	0.000498 (0.000156, 0.000841)
Cholesterol in VLDL particles (mmol·L^{-1})	0.00856 (–0.00208, 0.0192)	0.00376 (–0.00646, 0.014)
Cholesterol in LDL particles (mmol·L^{-1})	–0.0118 (–0.033, 0.00945)	–0.0186 (–0.0395, 0.00222)
Cholesterol in HDL particles (mmol·L^{-1})	–0.0113 (–0.0219, –0.000717)	–0.00606 (–0.0161, 0.004)
Total triglycerides concentration (mmol·L^{-1})	0.0164 (0.000393, 0.0323)	0.0127 (–0.00147, 0.027)
Triglycerides in CM particles (mmol·L^{-1})	0.000908 (0.000166, 0.00165)	0.000783 (0.0000966, 0.00147)
Triglycerides in VLDL particles (mmol·L^{-1})	0.0143 (0.00264, 0.026)	0.00949 (–0.00205, 0.021)
Triglycerides in LDL particles (mmol·L^{-1})	0.00093 (–0.000504, 0.00236)	0.000418 (–0.000985, 0.00182)
Triglycerides in HDL particles (mmol·L^{-1})	0.00127 (–0.000285, 0.00283)	0.001 (–0.000555, 0.00255)
Mean diameter for VLDL particles (nm)	0.174 (0.0779, 0.271)	0.142 (0.0357, 0.248)

Mean diameter for LDL particles (nm)	–0.00782 (–0.0134, –0.00228)	–0.00605 (–0.0115, –0.000603)
Mean diameter for HDL particles (nm)	–0.00535 (–0.0141, 0.00341)	–0.000257 (–0.0084, 0.00789)

Each regression analysis was adjusted for monitor wear time, parents' education, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Supplementary table 4. Associations for an isotemporal substitution of 30 minutes' MVPA for 30 minutes' sedentary time and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.0349 (–0.05, –0.0198)	–0.0263 (–0.0409, –0.0118)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.154 (–0.232, –0.0769)	–0.137 (–0.188, –0.0867)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.694 (–1.05, –0.339)	–0.577 (–0.841, –0.312)
Concentration of VLDL L3 particles (nmol·L^{-1})	–2.24 (–3.12, –1.36)	–1.47 (–2.32, –0.621)
Concentration of VLDL M particles (nmol·L^{-1})	–2.27 (–3.4, –1.14)	–1.49 (–2.61, –0.376)
Concentration of VLDL S particles (nmol·L^{-1})	0.127 (–0.849, 1.1)	0.469 (–0.518, 1.46)
Concentration of LDL L particles (nmol·L^{-1})	0.356 (–3.59, 4.3)	1.94 (–2.05, 5.92)
Concentration of LDL M particles (nmol·L^{-1})	–7.37 (–16.2, 1.46)	–1.76 (–10.5, 7.01)
Concentration of LDL S particles (nmol·L^{-1})	–6.57 (–10.9, –2.25)	–3.15 (–7.38, 1.08)
Concentration of LDL VS particles (nmol·L^{-1})	–2.85 (–4.96, –0.732)	–1.36 (–3.45, 0.734)
Concentration of HDL VL particles (nmol·L^{-1})	10.5 (2.75, 18.2)	3.51 (–3.95, 11)
Concentration of HDL L particles (nmol·L^{-1})	110 (48.3, 172)	47.9 (–11, 107)
Concentration of HDL M particles (nmol·L^{-1})	106 (56.2, 155)	75.2 (25.8, 125)
Concentration of HDL S particles (nmol·L^{-1})	–26 (–71.6, 19.5)	3.26 (–42, 48.5)
Concentration of HDL VS particles (nmol·L^{-1})	–20.4 (–48.5, 7.74)	–8.78 (–37.1, 19.6)
Total cholesterol concentration (mmol·L^{-1})	–0.024 (–0.0833, 0.0352)	0.000206 (–0.0595, 0.06)
Cholesterol in CM particles (mmol·L^{-1})	–0.00138 (–0.00213, –0.000617)	–0.00139 (–0.00206, –0.000729)
Cholesterol in VLDL particles (mmol·L^{-1})	–0.0381 (–0.0599, –0.0164)	–0.0205 (–0.0417, 0.000755)
Cholesterol in LDL particles (mmol·L^{-1})	–0.03 (–0.0735, 0.0134)	–0.00477 (–0.0481, 0.0386)
Cholesterol in HDL particles (mmol·L^{-1})	0.0423 (0.0208, 0.0639)	0.023 (0.00215, 0.0439)
Total triglycerides concentration (mmol·L^{-1})	–0.0543 (–0.0844, –0.0243)	–0.0476 (–0.0742, –0.0209)
Triglycerides in CM particles (mmol·L^{-1})	–0.00312 (–0.00477, –0.00147)	–0.00276 (–0.00421, –0.00132)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.0459 (–0.0802, –0.0116)	–0.0392 (–0.0599, –0.0185)
Triglycerides in LDL particles (mmol·L^{-1})	–0.00265 (–0.00559, 0.000294)	–0.000711 (–0.00363, 0.00221)
Triglycerides in HDL particles (mmol·L^{-1})	–0.00309 (–0.00628, 0.0000993)	–0.0021 (–0.00533, 0.00113)

Mean diameter for VLDL particles (nm)	–0.504 (–0.712, –0.297)	–0.446 (–0.637, –0.254)
Mean diameter for LDL particles (nm)	0.0266 (0.0153, 0.0379)	0.0203 (0.00907, 0.0316)
Mean diameter for HDL particles (nm)	0.0336 (0.0158, 0.0515)	0.0148 (–0.00214, 0.0317)

Each regression analysis was adjusted for monitor wear time, parents' education, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Supplementary table 5. Cross-sectional associations between aerobic fitness and serum lipoprotein measures

Lipoprotein measure	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0725	–0.1033	–0.0418	1.56E–05
VLDL L1 PN (nmol·L^{-1})	–0.2610	–0.3640	–0.1581	4.50E–06
VLDL L2 PN (nmol·L^{-1})	–0.9500	–1.3385	–0.5615	8.60E–06
VLDL L3 PN (nmol·L^{-1})	–2.5867	–3.4177	–1.7557	6.27E–08
VLDL M PN (nmol·L^{-1})	–2.7617	–3.7546	–1.7689	7.46E–07
VLDL S PN (nmol·L^{-1})	–1.2806	–2.3419	–0.2193	1.89E–02
LDL L PN (nmol·L^{-1})	–5.2875	–9.7038	–0.8712	1.98E–02
LDL M PN (nmol·L^{-1})	–17.2985	–26.3931	–8.2039	3.47E–04
LDL S PN (nmol·L^{-1})	–10.3361	–14.4806	–6.1916	6.08E–06
LDL VS PN (nmol·1^{-1})	–4.6706	–6.6237	–2.7174	1.26E–05
HDL VL PN (nmol·L^{-1})	17.3215	11.1888	23.4543	5.44E–07
HDL L PN (nmol·L^{-1})	163.2154	116.6032	209.8275	3.28E–09
HDL M PN (nmol·L^{-1})	88.5990	58.5261	118.6719	2.19E–07
HDL S PN (nmol·L^{-1})	–82.9860	–128.6851	–37.2869	6.00E–04
HDL VS PN (nmol·L^{-1})	–39.3204	–60.7217	–17.9191	5.25E–04
CM C (mmol·L^{-1})	–0.0028	–0.0039	–0.0016	1.74E–05
VLDL C (mmol·L^{-1})	–0.0598	–0.0791	–0.0405	6.64E–08
VLDL L1 C (mmol·L^{-1})	–0.0042	–0.0056	–0.0028	9.24E–08
VLDL L2 C (mmol·L^{-1})	–0.0090	–0.0124	–0.0057	1.41E–06
VLDL L3 C (mmol·L^{-1})	–0.0218	–0.0283	–0.0152	1.30E–08
VLDL M C (mmol·L^{-1})	–0.0169	–0.0223	–0.0116	3.97E–08
VLDL S C (mmol·L^{-1})	–0.0050	–0.0101	0.0001	5.39E–02
LDL C (mmol·L^{-1})	–0.0806	–0.1248	–0.0364	5.70E–04
LDL L C (mmol·L^{-1})	–0.0133	–0.0284	0.0018	8.36E–02
LDL M C (mmol·L^{-1})	–0.0350	–0.0552	–0.0149	9.86E–04
LDL S C (mmol·L^{-1})	–0.0189	–0.0267	–0.0111	9.80E–06
LDL VS C (mmol·L^{-1})	–0.0066	–0.0095	–0.0037	3.00E–05
HDL C (mmol·L^{-1})	0.0518	0.0371	0.0665	2.71E–09
HDL VL C (mmol·L^{-1})	0.0066	0.0044	0.0088	1.46E–07
HDL L C (mmol·L^{-1})	0.0357	0.0255	0.0459	3.12E–09
HDL M C (mmol·L^{-1})	0.0141	0.0098	0.0185	1.86E–08
HDL S C (mmol·L^{-1})	–0.0037	–0.0067	–0.0006	1.97E–02
HDL VS C (mmol·L^{-1})	–0.0013	–0.0023	–0.0002	1.81E–02
Total C (mmol·L^{-1})	–0.0899	–0.1471	–0.0328	2.60E–03
Non-HDL C (mmol·L^{-1})	–0.1418	–0.2004	–0.0832	1.03E–05
CM TG (mmol·L^{-1})	–0.0081	–0.0115	–0.0047	1.43E–05
VLDL TG (mmol·L^{-1})	–0.0683	–0.0947	–0.0419	3.11E–06
VLDL L1 TG (mmol·L^{-1})	–0.0106	–0.0150	–0.0062	1.02E–05

VLDL L2 TG (mmol·L^{-1})	–0.0212	–0.0299	–0.0124	1.03E–05
VLDL L3 TG (mmol·L^{-1})	–0.0249	–0.0344	–0.0154	2.51E–06
VLDL M TG (mmol·L^{-1})	–0.0096	–0.0135	–0.0056	9.46E–06
VLDL S TG (mmol·L^{-1})	–0.0021	–0.0032	–0.0009	7.31E–04
LDL TG (mmol·L^{-1})	–0.0059	–0.0089	–0.0029	2.48E–04
LDL L TG (mmol·L^{-1})	–0.0023	–0.0039	–0.0007	5.68E–03
LDL M TG (mmol·L^{-1})	–0.0018	–0.0030	–0.0006	3.08E–03
LDL S TG (mmol·L^{-1})	–0.0010	–0.0014	–0.0006	2.57E–05
LDL VS TG (mmol·L^{-1})	–0.0006	–0.0009	–0.0004	6.92E–06
HDL TG (mmol·L^{-1})	–0.0043	–0.0071	–0.0014	4.21E–03
HDL VL TG (mmol·L^{-1})	0.0000	–0.0002	0.0002	8.92E–01
HDL L TG (mmol·L^{-1})	0.0011	0.0004	0.0018	2.44E–03
HDL M TG (mmol·L^{-1})	–0.0022	–0.0033	–0.0010	3.42E–04
HDL S TG (mmol·L^{-1})	–0.0021	–0.0028	–0.0013	9.45E–07
HDL VS TG (mmol·L^{-1})	–0.0007	–0.0009	–0.0005	8.93E–08
Total TG (mmol·L^{-1})	–0.0854	–0.1188	–0.0521	3.70E–06
VLDL size (nm)	–0.6217	–0.8875	–0.3558	1.83E–05
LDL size (nm)	0.0160	0.0043	0.0276	8.02E–03
HDL size (nm)	0.0511	0.0363	0.0659	5.10E–09

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (103 m).
Adjusted for baseline values of age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 6. Cross-sectional associations between aerobic fitness and serum lipoprotein measures adjusted for waist circumference

Lipoprotein measure	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0383	–0.0704	–0.0061	2.05E–02
VLDL L1 PN (nmol·L^{-1})	–0.1383	–0.2468	–0.0298	1.34E–02
VLDL L2 PN (nmol·L^{-1})	–0.5032	–0.9171	–0.0892	1.81E–02
VLDL L3 PN (nmol·L^{-1})	–1.3781	–2.2431	–0.5131	2.32E–03
VLDL M PN (nmol·L^{-1})	–1.5510	–2.5701	–0.5318	3.51E–03
VLDL S PN (nmol·L^{-1})	–0.9652	–2.0340	0.1037	7.58E–02
LDL L PN (nmol·L^{-1})	–3.5544	–7.7373	0.6286	9.43E–02
LDL M PN (nmol·L^{-1})	–9.3085	–17.5958	–1.0212	2.84E–02
LDL S PN (nmol·L^{-1})	–5.0986	–8.9389	–1.2584	1.02E–02
LDL VS PN (nmol·1^{-1})	–2.4665	–4.2910	–0.6421	8.95E–03
HDL VL PN (nmol·L^{-1})	5.3159	–1.5835	12.2152	1.28E–01
HDL L PN (nmol·L^{-1})	55.7928	5.0649	106.5206	3.17E–02
HDL M PN (nmol·L^{-1})	26.0505	–11.2178	63.3188	1.67E–01
HDL S PN (nmol·L^{-1})	–42.5062	–90.7219	5.7095	8.28E–02
HDL VS PN (nmol·L^{-1})	–18.9440	–41.5894	3.7013	9.93E–02
CM C (mmol·L^{-1})	–0.0015	–0.0027	–0.0002	1.99E–02
VLDL C (mmol·L^{-1})	–0.0337	–0.0518	–0.0156	4.52E–04
VLDL L1 C (mmol·L^{-1})	–0.0022	–0.0036	–0.0008	3.16E–03
VLDL L2 C (mmol·L^{-1})	–0.0049	–0.0084	–0.0013	8.24E–03
VLDL L3 C (mmol·L^{-1})	–0.0118	–0.0183	–0.0053	5.60E–04
VLDL M C (mmol·L^{-1})	–0.0089	–0.0139	–0.0038	8.56E–04
VLDL S C (mmol·L^{-1})	–0.0044	–0.0095	0.0007	9.21E–02
LDL C (mmol·L^{-1})	–0.0465	–0.0870	–0.0060	2.53E–02
LDL L C (mmol·L^{-1})	–0.0111	–0.0255	0.0033	1.29E–01
LDL M C (mmol·L^{-1})	–0.0199	–0.0386	–0.0012	3.71E–02
LDL S C (mmol·L^{-1})	–0.0096	–0.0168	–0.0023	1.04E–02
LDL VS C (mmol·L^{-1})	–0.0035	–0.0062	–0.0008	1.25E–02
HDL C (mmol·L^{-1})	0.0170	–0.0001	0.0341	5.15E–02
HDL VL C (mmol·L^{-1})	0.0021	–0.0004	0.0046	9.14E–02
HDL L C (mmol·L^{-1})	0.0127	0.0017	0.0238	2.47E–02
HDL M C (mmol·L^{-1})	0.0048	–0.0005	0.0100	7.50E–02
HDL S C (mmol·L^{-1})	–0.0019	–0.0051	0.0014	2.60E–01
HDL VS C (mmol·L^{-1})	–0.0006	–0.0017	0.0005	2.67E–01
Total C (mmol·L^{-1})	–0.0638	–0.1174	–0.0101	2.08E–02
Non-HDL C (mmol·L^{-1})	–0.0807	–0.1338	–0.0277	3.49E–03
CM TG (mmol·L^{-1})	–0.0043	–0.0078	–0.0007	2.03E–02
VLDL TG (mmol·L^{-1})	–0.0365	–0.0644	–0.0085	1.14E–02
VLDL L1 TG (mmol·L^{-1})	–0.0056	–0.0102	–0.0010	1.89E–02

VLDL L2 TG (mmol·L^{-1})	–0.0112	–0.0205	–0.0018	2.00E–02
VLDL L3 TG (mmol·L^{-1})	–0.0132	–0.0233	–0.0031	1.16E–02
VLDL M TG (mmol·L^{-1})	–0.0053	–0.0094	–0.0011	1.39E–02
VLDL S TG (mmol·L^{-1})	–0.0012	–0.0024	–0.0001	4.07E–02
LDL TG (mmol·L^{-1})	–0.0030	–0.0058	–0.0002	3.81E–02
LDL L TG (mmol·L^{-1})	–0.0014	–0.0030	0.0003	1.02E–01
LDL M TG (mmol·L^{-1})	–0.0009	–0.0020	0.0003	1.35E–01
LDL S TG (mmol·L^{-1})	–0.0006	–0.0010	–0.0001	1.85E–02
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0001	1.02E–02
HDL TG (mmol·L^{-1})	–0.0031	–0.0060	–0.0001	4.38E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	3.23E–01
HDL L TG (mmol·L^{-1})	0.0001	–0.0006	0.0009	7.27E–01
HDL M TG (mmol·L^{-1})	–0.0013	–0.0025	–0.0001	2.86E–02
HDL S TG (mmol·L^{-1})	–0.0011	–0.0019	–0.0003	7.23E–03
HDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0001	4.34E–03
Total TG (mmol·L^{-1})	–0.0465	–0.0816	–0.0115	1.02E–02
VLDL size (nm)	–0.3142	–0.6008	–0.0275	3.23E–02
LDL size (nm)	0.0031	–0.0103	0.0166	6.41E–01
HDL size (nm)	0.0193	0.0038	0.0348	1.54E–02

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (103 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 7. Prospective associations between baseline aerobic fitness and follow-up serum lipoprotein measures

Lipoprotein measure	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0498	–0.0774	–0.0222	6.53E–04
VLDL L1 PN (nmol·L^{-1})	–0.1726	–0.2620	–0.0832	2.90E–04
VLDL L2 PN (nmol·L^{-1})	–0.6587	–0.9860	–0.3314	1.69E–04
VLDL L3 PN (nmol·L^{-1})	–1.3949	–2.0874	–0.7025	1.67E–04
VLDL M PN (nmol·L^{-1})	–1.6103	–2.3781	–0.8425	9.62E–05
VLDL S PN (nmol·L^{-1})	–0.4554	–1.2054	0.2945	2.29E–01
LDL L PN (nmol·L^{-1})	–0.8675	–3.3150	1.5800	4.81E–01
LDL M PN (nmol·L^{-1})	–2.8380	–7.2707	1.5947	2.05E–01
LDL S PN (nmol·L^{-1})	–2.1551	–4.3897	0.0795	5.84E–02
LDL VS PN (nmol·1^{-1})	–1.1115	–2.1587	–0.0643	3.79E–02
HDL VL PN (nmol·L^{-1})	0.9199	–3.4390	5.2787	6.74E–01
HDL L PN (nmol·L^{-1})	6.4885	–28.2483	41.2253	7.10E–01
HDL M PN (nmol·L^{-1})	18.5158	–13.4459	50.4775	2.51E–01
HDL S PN (nmol·L^{-1})	–24.6034	–63.7032	14.4964	2.13E–01
HDL VS PN (nmol·L^{-1})	–16.8579	–31.9547	–1.7610	2.93E–02
CM C (mmol·L^{-1})	–0.0019	–0.0029	–0.0009	4.11E–04
VLDL C (mmol·L^{-1})	–0.0194	–0.0326	–0.0061	4.86E–03
VLDL L1 C (mmol·L^{-1})	–0.0024	–0.0036	–0.0012	2.33E–04
VLDL L2 C (mmol·L^{-1})	–0.0057	–0.0085	–0.0029	1.71E–04
VLDL L3 C (mmol·L^{-1})	–0.0080	–0.0123	–0.0036	5.57E–04
VLDL M C (mmol·L^{-1})	–0.0064	–0.0105	–0.0022	3.07E–03
VLDL S C (mmol·L^{-1})	–0.0015	–0.0051	0.0021	4.11E–01
LDL C (mmol·L^{-1})	–0.0157	–0.0372	0.0058	1.49E–01
LDL L C (mmol·L^{-1})	–0.0030	–0.0121	0.0061	5.10E–01
LDL M C (mmol·L^{-1})	–0.0066	–0.0167	0.0034	1.92E–01
LDL S C (mmol·L^{-1})	–0.0042	–0.0083	–0.0001	4.37E–02
LDL VS C (mmol·L^{-1})	–0.0017	–0.0032	–0.0001	3.39E–02
HDL C (mmol·L^{-1})	0.0046	–0.0074	0.0165	4.46E–01
HDL VL C (mmol·L^{-1})	0.0004	–0.0012	0.0019	6.59E–01
HDL L C (mmol·L^{-1})	0.0016	–0.0059	0.0091	6.73E–01
HDL M C (mmol·L^{-1})	0.0023	–0.0019	0.0064	2.81E–01
HDL S C (mmol·L^{-1})	–0.0012	–0.0037	0.0013	3.31E–01
HDL VS C (mmol·L^{-1})	–0.0007	–0.0014	0.0000	4.17E–02
Total C (mmol·L^{-1})	–0.0237	–0.0507	0.0033	8.43E–02
Non-HDL C (mmol·L^{-1})	–0.0231	–0.0475	0.0012	6.23E–02
CM TG (mmol·L^{-1})	–0.0056	–0.0087	–0.0025	6.47E–04
VLDL TG (mmol·L^{-1})	–0.0444	–0.0672	–0.0216	2.56E–04
VLDL L1 TG (mmol·L^{-1})	–0.0072	–0.0110	–0.0034	3.63E–04

VLDL L2 TG (mmol·L^{-1})	–0.0145	–0.0219	–0.0070	2.61E–04
VLDL L3 TG (mmol·L^{-1})	–0.0161	–0.0242	–0.0080	1.99E–04
VLDL M TG (mmol·L^{-1})	–0.0067	–0.0101	–0.0033	2.05E–04
VLDL S TG (mmol·L^{-1})	–0.0011	–0.0021	–0.0002	1.90E–02
LDL TG (mmol·L^{-1})	–0.0013	–0.0033	0.0007	2.10E–01
LDL L TG (mmol·L^{-1})	–0.0008	–0.0020	0.0005	2.22E–01
LDL M TG (mmol·L^{-1})	–0.0002	–0.0011	0.0007	6.55E–01
LDL S TG (mmol·L^{-1})	–0.0006	–0.0010	–0.0002	3.28E–03
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0007	–0.0002	8.71E–04
HDL TG (mmol·L^{-1})	–0.0027	–0.0052	–0.0002	3.18E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0002	0.0001	4.83E–01
HDL L TG (mmol·L^{-1})	0.0001	–0.0005	0.0006	8.03E–01
HDL M TG (mmol·L^{-1})	–0.0013	–0.0023	–0.0003	1.37E–02
HDL S TG (mmol·L^{-1})	–0.0011	–0.0018	–0.0004	2.88E–03
HDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0002	2.05E–04
Total TG (mmol·L^{-1})	–0.0556	–0.0848	–0.0264	3.42E–04
VLDL size (nm)	–0.4369	–0.6642	–0.2096	3.06E–04
LDL size (nm)	0.0081	–0.0027	0.0190	1.40E–01
HDL size (nm)	0.0034	–0.0072	0.0141	5.20E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (102 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 8. Prospective associations between baseline aerobic fitness and follow-up serum lipoprotein measures adjusted for waist circumference

Lipoprotein measure	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0224	–0.0484	0.0035	8.90E–02
VLDL L1 PN (nmol·L^{-1})	–0.0774	–0.1581	0.0032	5.94E–02
VLDL L2 PN (nmol·L^{-1})	–0.3084	–0.5973	–0.0196	3.68E–02
VLDL L3 PN (nmol·L^{-1})	–0.6748	–1.3028	–0.0469	3.57E–02
VLDL M PN (nmol·L^{-1})	–0.8176	–1.5097	–0.1255	2.14E–02
VLDL S PN (nmol·L^{-1})	–0.2286	–1.0488	0.5916	5.79E–01
LDL L PN (nmol·L^{-1})	–0.4463	–3.1513	2.2587	7.42E–01
LDL M PN (nmol·L^{-1})	–1.3098	–6.5109	3.8913	6.16E–01
LDL S PN (nmol·L^{-1})	–1.0091	–3.6703	1.6521	4.51E–01
LDL VS PN (nmol·1^{-1})	–0.6381	–1.8771	0.6008	3.07E–01
HDL VL PN (nmol·L^{-1})	–1.8259	–6.6052	2.9534	4.47E–01
HDL L PN (nmol·L^{-1})	–14.2781	–52.7322	24.1760	4.60E–01
HDL M PN (nmol·L^{-1})	–6.0963	–39.3374	27.1448	7.15E–01
HDL S PN (nmol·L^{-1})	–8.9497	–47.9038	30.0043	6.47E–01
HDL VS PN (nmol·L^{-1})	–8.5965	–24.6609	7.4680	2.88E–01
CM C (mmol·L^{-1})	–0.0008	–0.0018	0.0001	7.84E–02
VLDL C (mmol·L^{-1})	–0.0103	–0.0237	0.0032	1.31E–01
VLDL L1 C (mmol·L^{-1})	–0.0011	–0.0022	0.0000	4.61E–02
VLDL L2 C (mmol·L^{-1})	–0.0026	–0.0051	–0.0001	4.18E–02
VLDL L3 C (mmol·L^{-1})	–0.0042	–0.0086	0.0002	5.90E–02
VLDL M C (mmol·L^{-1})	–0.0037	–0.0076	0.0003	6.73E–02
VLDL S C (mmol·L^{-1})	–0.0010	–0.0051	0.0031	6.19E–01
LDL C (mmol·L^{-1})	–0.0091	–0.0339	0.0156	4.63E–01
LDL L C (mmol·L^{-1})	–0.0021	–0.0120	0.0078	6.79E–01
LDL M C (mmol·L^{-1})	–0.0034	–0.0150	0.0082	5.64E–01
LDL S C (mmol·L^{-1})	–0.0021	–0.0069	0.0026	3.75E–01
LDL VS C (mmol·L^{-1})	–0.0010	–0.0027	0.0008	2.65E–01
HDL C (mmol·L^{-1})	–0.0061	–0.0182	0.0061	3.20E–01
HDL VL C (mmol·L^{-1})	–0.0008	–0.0025	0.0009	3.49E–01
HDL L C (mmol·L^{-1})	–0.0034	–0.0114	0.0045	3.88E–01
HDL M C (mmol·L^{-1})	–0.0017	–0.0059	0.0025	4.13E–01
HDL S C (mmol·L^{-1})	–0.0005	–0.0029	0.0020	7.12E–01
HDL VS C (mmol·L^{-1})	–0.0004	–0.0011	0.0003	2.81E–01
Total C (mmol·L^{-1})	–0.0218	–0.0541	0.0105	1.81E–01
Non-HDL C (mmol·L^{-1})	–0.0132	–0.0433	0.0170	3.86E–01
CM TG (mmol·L^{-1})	–0.0025	–0.0054	0.0004	9.20E–02
VLDL TG (mmol·L^{-1})	–0.0202	–0.0404	0.0001	5.09E–02
VLDL L1 TG (mmol·L^{-1})	–0.0032	–0.0067	0.0003	6.88E–02

VLDL L2 TG (mmol·L^{-1})	–0.0065	–0.0132	0.0002	5.63E–02
VLDL L3 TG (mmol·L^{-1})	–0.0073	–0.0145	–0.0001	4.62E–02
VLDL M TG (mmol·L^{-1})	–0.0032	–0.0062	–0.0002	3.91E–02
VLDL S TG (mmol·L^{-1})	–0.0005	–0.0014	0.0004	2.43E–01
LDL TG (mmol·L^{-1})	–0.0006	–0.0027	0.0015	5.63E–01
LDL L TG (mmol·L^{-1})	–0.0004	–0.0016	0.0008	5.12E–01
LDL M TG (mmol·L^{-1})	–0.0001	–0.0011	0.0009	8.80E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0008	0.0001	1.04E–01
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0005	0.0000	5.95E–02
HDL TG (mmol·L^{-1})	–0.0016	–0.0040	0.0008	1.81E–01
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	1.75E–01
HDL L TG (mmol·L^{-1})	–0.0003	–0.0009	0.0003	3.27E–01
HDL M TG (mmol·L^{-1})	–0.0006	–0.0016	0.0004	2.10E–01
HDL S TG (mmol·L^{-1})	–0.0004	–0.0010	0.0002	2.10E–01
HDL VS TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	4.65E–02
Total TG (mmol·L^{-1})	–0.0259	–0.0521	0.0003	5.27E–02
VLDL size (nm)	–0.1971	–0.4049	0.0107	6.26E–02
LDL size (nm)	0.0031	–0.0091	0.0154	6.08E–01
HDL size (nm)	–0.0034	–0.0149	0.0081	5.59E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (102 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, waist circumference, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 9. Cross-sectional associations between waist circumference and serum lipoprotein measures

Lipoprotein measure	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.1008	0.0664	0.1352	2.46E–07
VLDL L1 PN (nmol·L^{-1})	0.3559	0.2539	0.4579	3.57E–09
VLDL L2 PN (nmol·L^{-1})	1.2838	0.9226	1.6451	2.20E–09
VLDL L3 PN (nmol·L^{-1})	3.4820	2.7236	4.2404	8.68E–13
VLDL M PN (nmol·L^{-1})	3.5739	2.5615	4.5862	2.63E–09
VLDL S PN (nmol·L^{-1})	1.3280	0.5074	2.1485	2.00E–03
LDL L PN (nmol·L^{-1})	5.9392	2.5702	9.3083	8.35E–04
LDL M PN (nmol·L^{-1})	22.9680	14.9578	30.9783	3.95E–07
LDL S PN (nmol·L^{-1})	14.5329	10.6892	18.3767	3.89E–10
LDL VS PN (nmol·1^{-1})	6.2793	4.4282	8.1305	7.54E–09
HDL VL PN (nmol·L^{-1})	–30.9149	–36.5335	–25.2962	1.19E–15
HDL L PN (nmol·L^{-1})	–278.3540	–325.1463	–231.5618	5.46E–17
HDL M PN (nmol·L^{-1})	–153.0544	–192.9028	–113.2061	2.47E–10
HDL S PN (nmol·L^{-1})	114.1365	76.9297	151.3434	8.82E–08
HDL VS PN (nmol·L^{-1})	53.9795	35.9350	72.0240	1.56E–07
CM C (mmol·L^{-1})	0.0037	0.0025	0.0049	8.33E–08
VLDL C (mmol·L^{-1})	0.0781	0.0599	0.0962	7.44E–12
VLDL L1 C (mmol·L^{-1})	0.0057	0.0044	0.0071	1.83E–11
VLDL L2 C (mmol·L^{-1})	0.0121	0.0088	0.0154	1.34E–09
VLDL L3 C (mmol·L^{-1})	0.0288	0.0233	0.0343	7.71E–15
VLDL M C (mmol·L^{-1})	0.0232	0.0176	0.0288	2.25E–11
VLDL S C (mmol·L^{-1})	0.0041	0.0002	0.0080	3.98E–02
LDL C (mmol·L^{-1})	0.1017	0.0630	0.1404	2.32E–06
LDL L C (mmol·L^{-1})	0.0113	–0.0011	0.0236	7.25E–02
LDL M C (mmol·L^{-1})	0.0445	0.0269	0.0621	4.85E–06
LDL S C (mmol·L^{-1})	0.0261	0.0188	0.0334	1.83E–09
LDL VS C (mmol·L^{-1})	0.0088	0.0061	0.0116	2.81E–08
HDL C (mmol·L^{-1})	–0.0903	–0.1064	–0.0742	5.55E–16
HDL VL C (mmol·L^{-1})	–0.0116	–0.0136	–0.0095	7.89E–16
HDL L C (mmol·L^{-1})	–0.0604	–0.0703	–0.0504	2.45E–17
HDL M C (mmol·L^{-1})	–0.0235	–0.0288	–0.0182	2.84E–12
HDL S C (mmol·L^{-1})	0.0050	0.0024	0.0076	3.55E–04
HDL VS C (mmol·L^{-1})	0.0016	0.0006	0.0027	1.99E–03
Total C (mmol·L^{-1})	0.0921	0.0430	0.1411	4.11E–04
Non-HDL C (mmol·L^{-1})	0.1823	0.1352	0.2295	2.00E–10
CM TG (mmol·L^{-1})	0.0114	0.0074	0.0153	3.24E–07
VLDL TG (mmol·L^{-1})	0.0922	0.0675	0.1170	6.16E–10
VLDL L1 TG (mmol·L^{-1})	0.0145	0.0101	0.0189	1.68E–08

VLDL L2 TG (mmol·L^{-1})	0.0288	0.0207	0.0368	2.06E–09
VLDL L3 TG (mmol·L^{-1})	0.0337	0.0251	0.0422	1.27E–10
VLDL M TG (mmol·L^{-1})	0.0126	0.0089	0.0163	7.00E–09
VLDL S TG (mmol·L^{-1})	0.0026	0.0016	0.0037	5.23E–06
LDL TG (mmol·L^{-1})	0.0083	0.0056	0.0110	1.15E–07
LDL L TG (mmol·L^{-1})	0.0029	0.0017	0.0041	1.14E–05
LDL M TG (mmol·L^{-1})	0.0027	0.0016	0.0037	3.81E–06
LDL S TG (mmol·L^{-1})	0.0013	0.0008	0.0019	3.93E–06
LDL VS TG (mmol·L^{-1})	0.0009	0.0006	0.0012	4.36E–07
HDL TG (mmol·L^{-1})	0.0046	0.0011	0.0081	1.02E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0004	0.0001	2.45E–01
HDL L TG (mmol·L^{-1})	–0.0022	–0.0031	–0.0014	2.05E–06
HDL M TG (mmol·L^{-1})	0.0027	0.0013	0.0041	3.45E–04
HDL S TG (mmol·L^{-1})	0.0029	0.0020	0.0038	2.65E–08
HDL VS TG (mmol·L^{-1})	0.0010	0.0007	0.0012	6.06E–11
Total TG (mmol·L^{-1})	0.1143	0.0808	0.1479	6.45E–09
VLDL size (nm)	0.8731	0.6303	1.1159	1.60E–09
LDL size (nm)	–0.0299	–0.0408	–0.0189	1.13E–06
HDL size (nm)	–0.0831	–0.0972	–0.0690	8.22E–17

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of waist circumference (7.5 cm).
Adjusted for baseline values of age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 10. Prospective associations between baseline waist circumference and follow-up serum lipoprotein measures

Lipoprotein measure	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0783	0.0440	0.1126	2.73E–05
VLDL L1 PN (nmol·L^{-1})	0.2670	0.1587	0.3753	7.44E–06
VLDL L2 PN (nmol·L^{-1})	0.9721	0.5842	1.3599	5.55E–06
VLDL L3 PN (nmol·L^{-1})	2.0129	1.1881	2.8378	8.92E–06
VLDL M PN (nmol·L^{-1})	2.1247	1.1988	3.0506	2.49E–05
VLDL S PN (nmol·L^{-1})	0.5890	–0.1824	1.3604	1.32E–01
LDL L PN (nmol·L^{-1})	1.1370	–0.9853	3.2593	2.88E–01
LDL M PN (nmol·L^{-1})	4.0986	–1.3065	9.5038	1.34E–01
LDL S PN (nmol·L^{-1})	3.0830	–0.2160	6.3821	6.64E–02
LDL VS PN (nmol·1^{-1})	1.3421	–0.2155	2.8997	8.99E–02
HDL VL PN (nmol·L^{-1})	–5.9558	–10.9209	–0.9907	1.96E–02
HDL L PN (nmol·L^{-1})	–47.3548	–84.8384	–9.8711	1.42E–02
HDL M PN (nmol·L^{-1})	–51.6325	–86.8176	–16.4473	4.77E–03
HDL S PN (nmol·L^{-1})	39.6368	–0.6107	79.8842	5.35E–02
HDL VS PN (nmol·L^{-1})	20.6371	–0.6858	41.9599	5.76E–02
CM C (mmol·L^{-1})	0.0029	0.0016	0.0041	1.87E–05
VLDL C (mmol·L^{-1})	0.0254	0.0098	0.0410	1.87E–03
VLDL L1 C (mmol·L^{-1})	0.0036	0.0022	0.0051	6.80E–06
VLDL L2 C (mmol·L^{-1})	0.0085	0.0050	0.0119	7.24E–06
VLDL L3 C (mmol·L^{-1})	0.0102	0.0045	0.0159	6.68E–04
VLDL M C (mmol·L^{-1})	0.0077	0.0033	0.0121	9.45E–04
VLDL S C (mmol·L^{-1})	0.0015	–0.0020	0.0049	3.97E–01
LDL C (mmol·L^{-1})	0.0189	–0.0069	0.0447	1.48E–01
LDL L C (mmol·L^{-1})	0.0029	–0.0053	0.0112	4.80E–01
LDL M C (mmol·L^{-1})	0.0088	–0.0031	0.0206	1.43E–01
LDL S C (mmol·L^{-1})	0.0058	–0.0001	0.0117	5.50E–02
LDL VS C (mmol·L^{-1})	0.0020	–0.0002	0.0041	6.93E–02
HDL C (mmol·L^{-1})	–0.0241	–0.0374	–0.0109	5.91E–04
HDL VL C (mmol·L^{-1})	–0.0025	–0.0044	–0.0007	8.69E–03
HDL L C (mmol·L^{-1})	–0.0115	–0.0199	–0.0031	8.08E–03
HDL M C (mmol·L^{-1})	–0.0086	–0.0133	–0.0040	4.99E–04
HDL S C (mmol·L^{-1})	0.0020	–0.0005	0.0044	1.20E–01
HDL VS C (mmol·L^{-1})	0.0008	–0.0002	0.0017	1.07E–01
Total C (mmol·L^{-1})	0.0115	–0.0192	0.0422	4.55E–01
Non-HDL C (mmol·L^{-1})	0.0269	–0.0061	0.0598	1.08E–01
CM TG (mmol·L^{-1})	0.0088	0.0049	0.0127	2.95E–05
VLDL TG (mmol·L^{-1})	0.0668	0.0401	0.0935	5.84E–06
VLDL L1 TG (mmol·L^{-1})	0.0112	0.0066	0.0158	9.21E–06

VLDL L2 TG (mmol·L^{-1})	0.0220	0.0132	0.0308	5.66E–06
VLDL L3 TG (mmol·L^{-1})	0.0241	0.0146	0.0335	4.23E–06
VLDL M TG (mmol·L^{-1})	0.0092	0.0054	0.0130	9.14E–06
VLDL S TG (mmol·L^{-1})	0.0015	0.0005	0.0025	3.97E–03
LDL TG (mmol·L^{-1})	0.0018	–0.0006	0.0041	1.33E–01
LDL L TG (mmol·L^{-1})	0.0010	–0.0002	0.0021	1.05E–01
LDL M TG (mmol·L^{-1})	0.0003	–0.0005	0.0012	4.31E–01
LDL S TG (mmol·L^{-1})	0.0008	0.0003	0.0013	2.97E–03
LDL VS TG (mmol·L^{-1})	0.0006	0.0003	0.0009	2.92E–04
HDL TG (mmol·L^{-1})	0.0032	0.0001	0.0062	4.06E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	3.21E–01
HDL L TG (mmol·L^{-1})	–0.0009	–0.0015	–0.0002	1.61E–02
HDL M TG (mmol·L^{-1})	0.0019	0.0007	0.0031	2.12E–03
HDL S TG (mmol·L^{-1})	0.0018	0.0011	0.0026	1.91E–05
HDL VS TG (mmol·L^{-1})	0.0007	0.0004	0.0009	2.33E–06
Total TG (mmol·L^{-1})	0.0828	0.0483	0.1173	1.18E–05
VLDL size (nm)	0.6618	0.3968	0.9268	5.94E–06
LDL size (nm)	–0.0120	–0.0233	–0.0006	3.96E–02
HDL size (nm)	–0.0160	–0.0278	–0.0042	8.92E–03

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of waist circumference (7.5 cm).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 11. Cross-sectional associations between waist circumference and aerobic fitness

Measure	Coefficient	Lower CI	Upper CI	*p* value
Andersen test (m)	–40.1	–45.4	–34.8	2.23E–21

Regression coefficients are in metres run per standard deviation of waist circumference (7.4 cm).
Adjusted for baseline values of age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a threshold of 0.05.
In notation of *p* value 1.23E–02 stands for '1.23 times 10 to the power of –02' or 0.0123.
Abbreviations: CI = confidence interval.

Supplementary table 12. Prospective associations between baseline waist circumference and follow-up aerobic fitness

Measure	Coefficient	Lower CI	Upper CI	*p* value
Andersen test (m)	–16.7	–21.6	–11.8	6.68E–09

Regression coefficients are in metres run per standard deviation of waist circumference (7.4 cm).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and aerobic fitness. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a threshold of 0.05.
In notation of *p* value 1.23E–02 stands for '1.23 times 10 to the power of –02' or 0.0123.
Abbreviations: CI = confidence interval.

Supplementary table 13. Associations between baseline vigorous-intensity physical activity (VPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0439	–0.0652	–0.0227	1.17E–04	–0.0304	–0.0468	–0.0140	4.71E–04
VLDL L1 PN (nmol·L^{-1})	–0.1318	–0.1983	–0.0653	2.06E–04	–0.0860	–0.1411	–0.0309	2.79E–03
VLDL L2 PN (nmol·L^{-1})	–0.4809	–0.7212	–0.2406	1.82E–04	–0.3136	–0.5226	–0.1047	3.95E–03
VLDL L3 PN (nmol·L^{-1})	–0.9019	–1.3944	–0.4095	5.44E–04	–0.5655	–1.0230	–0.1080	1.63E–02
VLDL M PN (nmol·L^{-1})	–0.8315	–1.5453	–0.1176	2.32E–02	–0.4367	–1.2215	0.3481	2.70E–01
VLDL S PN (nmol·L^{-1})	–0.1670	–0.7663	0.4322	5.79E–01	–0.0384	–0.6950	0.6183	9.07E–01
LDL L PN (nmol·L^{-1})	–1.1285	–3.0452	0.7881	2.43E–01	–0.9264	–2.9731	1.1202	3.68E–01
LDL M PN (nmol·L^{-1})	–4.5116	–9.7008	0.6776	8.71E–02	–3.8345	–9.1844	1.5155	1.57E–01
LDL S PN (nmol·L^{-1})	–2.8130	–5.8236	0.1976	6.65E–02	–2.3074	–5.2987	0.6840	1.28E–01
LDL VS PN (nmol·1^{-1})	–1.3579	–2.8924	0.1767	8.17E–02	–1.1370	–2.6677	0.3937	1.42E–01
HDL VL PN (nmol·L^{-1})	2.8404	–2.6701	8.3509	3.06E–01	1.7695	–3.6925	7.2314	5.19E–01
HDL L PN (nmol·L^{-1})	25.3609	–14.5915	65.3133	2.09E–01	17.7252	–22.0069	57.4572	3.75E–01
HDL M PN (nmol·L^{-1})	18.1486	–17.2347	53.5319	3.09E–01	8.6224	–25.6787	42.9235	6.17E–01
HDL S PN (nmol·L^{-1})	–18.4718	–50.9702	14.0266	2.60E–01	–10.4427	–42.6659	21.7806	5.19E–01
HDL VS PN (nmol·L^{-1})	–11.1428	–25.5859	3.3003	1.28E–01	–7.2357	–21.2975	6.8261	3.07E–01
CM C (mmol·L^{-1})	–0.0015	–0.0023	–0.0008	8.12E–05	–0.0010	–0.0016	–0.0005	7.39E–04
VLDL C (mmol·L^{-1})	–0.0148	–0.0243	–0.0052	3.01E–03	–0.0102	–0.0202	–0.0002	4.59E–02
VLDL L1 C (mmol·L^{-1})	–0.0018	–0.0027	–0.0009	1.23E–04	–0.0012	–0.0019	–0.0005	1.79E–03
VLDL L2 C (mmol·L^{-1})	–0.0041	–0.0062	–0.0020	2.57E–04	–0.0026	–0.0045	–0.0007	8.22E–03
VLDL L3 C (mmol·L^{-1})	–0.0058	–0.0094	–0.0021	2.58E–03	–0.0040	–0.0074	–0.0007	1.98E–02
VLDL M C (mmol·L^{-1})	–0.0048	–0.0086	–0.0011	1.27E–02	–0.0034	–0.0074	0.0005	8.63E–02
VLDL S C (mmol·L^{-1})	–0.0008	–0.0033	0.0018	5.43E–01	–0.0005	–0.0033	0.0023	7.39E–01
LDL C (mmol·L^{-1})	–0.0220	–0.0473	0.0033	8.65E–02	–0.0189	–0.0449	0.0072	1.53E–01
LDL L C (mmol·L^{-1})	–0.0030	–0.0101	0.0042	4.11E–01	–0.0024	–0.0100	0.0052	5.25E–01

LDL M C (mmol·L^{-1})	–0.0092	–0.0206	0.0023	1.16E–01	–0.0076	–0.0195	0.0042	2.01E–01
LDL S C (mmol·L^{-1})	–0.0054	–0.0109	0.0001	5.58E–02	–0.0044	–0.0099	0.0011	1.15E–01
LDL VS C (mmol·L^{-1})	–0.0020	–0.0042	0.0001	6.63E–02	–0.0017	–0.0039	0.0005	1.28E–01
HDL C (mmol·L^{-1})	0.0082	–0.0079	0.0242	3.12E–01	0.0041	–0.0116	0.0198	6.02E–01
HDL VL C (mmol·L^{-1})	0.0010	–0.0011	0.0030	3.53E–01	0.0005	–0.0015	0.0025	6.22E–01
HDL L C (mmol·L^{-1})	0.0053	–0.0032	0.0138	2.13E–01	0.0035	–0.0049	0.0119	4.07E–01
HDL M C (mmol·L^{-1})	0.0026	–0.0023	0.0076	2.90E–01	0.0011	–0.0038	0.0060	6.65E–01
HDL S C (mmol·L^{-1})	–0.0009	–0.0030	0.0013	4.34E–01	–0.0004	–0.0027	0.0018	6.94E–01
HDL VS C (mmol·L^{-1})	–0.0005	–0.0012	0.0003	2.48E–01	–0.0003	–0.0011	0.0005	4.48E–01
Total C (mmol·L^{-1})	–0.0242	–0.0567	0.0083	1.42E–01	–0.0231	–0.0566	0.0105	1.74E–01
Non-HDL C (mmol·L^{-1})	–0.0313	–0.0575	–0.0050	2.05E–02	–0.0271	–0.0528	–0.0014	3.93E–02
CM TG (mmol·L^{-1})	–0.0051	–0.0075	–0.0027	8.23E–05	–0.0036	–0.0054	–0.0018	2.30E–04
VLDL TG (mmol·L^{-1})	–0.0309	–0.0479	–0.0139	5.93E–04	–0.0193	–0.0348	–0.0037	1.60E–02
VLDL L1 TG (mmol·L^{-1})	–0.0060	–0.0089	–0.0032	8.48E–05	–0.0041	–0.0064	–0.0018	6.38E–04
VLDL L2 TG (mmol·L^{-1})	–0.0111	–0.0166	–0.0056	1.51E–04	–0.0074	–0.0120	–0.0027	2.42E–03
VLDL L3 TG (mmol·L^{-1})	–0.0110	–0.0170	–0.0050	5.56E–04	–0.0068	–0.0124	–0.0013	1.66E–02
VLDL M TG (mmol·L^{-1})	–0.0036	–0.0064	–0.0009	1.12E–02	–0.0019	–0.0049	0.0011	2.05E–01
VLDL S TG (mmol·L^{-1})	–0.0004	–0.0014	0.0006	4.46E–01	–0.0001	–0.0011	0.0010	9.11E–01
LDL TG (mmol·L^{-1})	–0.0012	–0.0031	0.0008	2.44E–01	–0.0009	–0.0028	0.0011	3.78E–01
LDL L TG (mmol·L^{-1})	–0.0003	–0.0016	0.0009	5.95E–01	–0.0001	–0.0014	0.0011	8.26E–01
LDL M TG (mmol·L^{-1})	–0.0003	–0.0012	0.0005	4.26E–01	–0.0003	–0.0011	0.0005	4.83E–01
LDL S TG (mmol·L^{-1})	–0.0007	–0.0010	–0.0003	4.40E–04	–0.0005	–0.0008	–0.0002	8.12E–04
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0002	4.51E–05	–0.0003	–0.0004	–0.0002	4.20E–05
HDL TG (mmol·L^{-1})	–0.0014	–0.0037	0.0009	2.33E–01	–0.0008	–0.0032	0.0017	5.43E–01
HDL VL TG (mmol·L^{-1})	0.0000	–0.0001	0.0001	8.32E–01	0.0000	–0.0002	0.0001	5.00E–01
HDL L TG (mmol·L^{-1})	0.0004	–0.0003	0.0010	2.40E–01	0.0002	–0.0004	0.0009	5.24E–01
HDL M TG (mmol·L^{-1})	–0.0007	–0.0018	0.0004	1.93E–01	–0.0003	–0.0015	0.0008	5.82E–01

HDL S TG (mmol·L^{-1})	–0.0007	–0.0014	0.0000	4.77E–02	–0.0004	–0.0011	0.0003	3.04E–01
HDL VS TG (mmol·L^{-1})	–0.0003	–0.0005	–0.0002	1.14E–04	–0.0002	–0.0004	–0.0001	1.24E–03
Total TG (mmol·L^{-1})	–0.0399	–0.0613	–0.0186	4.34E–04	–0.0254	–0.0447	–0.0061	1.07E–02
VLDL size (nm)	–0.3647	–0.5437	–0.1856	1.44E–04	–0.2539	–0.4038	–0.1041	1.27E–03
LDL size (nm)	0.0104	–0.0007	0.0215	6.59E–02	0.0084	–0.0028	0.0196	1.38E–01
HDL size (nm)	0.0077	–0.0029	0.0183	1.52E–01	0.0051	–0.0056	0.0157	3.45E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity.
Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 14. Associations between change in vigorous-intensity physical activity (VPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0108	–0.0042	0.0258	1.53E–01	0.0140	–0.0018	0.0299	8.21E–02
VLDL L1 PN (nmol·L^{-1})	0.0235	–0.0249	0.0719	3.35E–01	0.0345	–0.0189	0.0879	2.01E–01
VLDL L2 PN (nmol·L^{-1})	0.0966	–0.0874	0.2806	2.97E–01	0.1375	–0.0677	0.3428	1.85E–01
VLDL L3 PN (nmol·L^{-1})	–0.0185	–0.5147	0.4776	9.41E–01	0.0546	–0.4946	0.6039	8.43E–01
VLDL M PN (nmol·L^{-1})	–0.1108	–0.9120	0.6903	7.83E–01	–0.0480	–0.9019	0.8058	9.11E–01
VLDL S PN (nmol·L^{-1})	–0.4840	–1.2367	0.2687	2.03E–01	–0.4723	–1.2328	0.2883	2.19E–01
LDL L PN (nmol·L^{-1})	–0.7025	–2.7500	1.3449	4.95E–01	–0.6772	–2.7441	1.3896	5.14E–01
LDL M PN (nmol·L^{-1})	0.4207	–3.8552	4.6965	8.44E–01	0.5066	–3.8003	4.8135	8.15E–01
LDL S PN (nmol·L^{-1})	0.3272	–2.0146	2.6690	7.81E–01	0.3961	–1.9682	2.7604	7.38E–01
LDL VS PN (nmol·1^{-1})	0.0164	–1.1083	1.1411	9.77E–01	0.0496	–1.0897	1.1888	9.31E–01
HDL VL PN (nmol·L^{-1})	0.4299	–4.8948	5.7546	8.72E–01	0.2333	–5.0853	5.5518	9.30E–01
HDL L PN (nmol·L^{-1})	6.6720	–33.3875	46.7316	7.40E–01	4.8238	–35.1531	44.8007	8.10E–01
HDL M PN (nmol·L^{-1})	–6.9981	–43.5765	29.5802	7.03E–01	–9.3789	–45.9249	27.1672	6.09E–01
HDL S PN (nmol·L^{-1})	–2.5932	–33.9902	28.8037	8.69E–01	–1.3061	–33.1582	30.5460	9.35E–01
HDL VS PN (nmol·L^{-1})	2.0747	–13.7713	17.9207	7.94E–01	2.9408	–13.2640	19.1457	7.18E–01
CM C (mmol·L^{-1})	0.0003	–0.0003	0.0009	2.95E–01	0.0004	–0.0002	0.0010	1.85E–01
VLDL C (mmol·L^{-1})	–0.0051	–0.0181	0.0079	4.36E–01	–0.0045	–0.0182	0.0091	5.07E–01
VLDL L1 C (mmol·L^{-1})	0.0001	–0.0006	0.0008	7.62E–01	0.0003	–0.0006	0.0011	5.37E–01
VLDL L2 C (mmol·L^{-1})	0.0004	–0.0015	0.0022	6.69E–01	0.0007	–0.0013	0.0028	4.83E–01
VLDL L3 C (mmol·L^{-1})	–0.0014	–0.0054	0.0027	5.11E–01	–0.0010	–0.0055	0.0034	6.42E–01
VLDL M C (mmol·L^{-1})	–0.0018	–0.0068	0.0031	4.60E–01	–0.0016	–0.0067	0.0034	5.19E–01
VLDL S C (mmol·L^{-1})	–0.0018	–0.0051	0.0015	2.81E–01	–0.0018	–0.0051	0.0016	2.89E–01
LDL C (mmol·L^{-1})	0.0014	–0.0202	0.0231	8.97E–01	0.0018	–0.0201	0.0237	8.69E–01
LDL L C (mmol·L^{-1})	–0.0021	–0.0098	0.0056	5.90E–01	–0.0020	–0.0097	0.0058	6.09E–01

LDL M C (mmol·L^{-1})	0.0010	–0.0092	0.0111	8.51E–01	0.0012	–0.0091	0.0114	8.21E–01
LDL S C (mmol·L^{-1})	0.0008	–0.0036	0.0052	7.18E–01	0.0009	–0.0035	0.0054	6.79E–01
LDL VS C (mmol·L^{-1})	0.0001	–0.0016	0.0017	9.36E–01	0.0001	–0.0015	0.0018	8.93E–01
HDL C (mmol·L^{-1})	0.0022	–0.0141	0.0184	7.89E–01	0.0012	–0.0150	0.0174	8.80E–01
HDL VL C (mmol·L^{-1})	0.0001	–0.0018	0.0021	8.90E–01	0.0001	–0.0019	0.0020	9.56E–01
HDL L C (mmol·L^{-1})	0.0014	–0.0071	0.0099	7.44E–01	0.0010	–0.0075	0.0095	8.18E–01
HDL M C (mmol·L^{-1})	–0.0006	–0.0056	0.0045	8.23E–01	–0.0010	–0.0061	0.0041	7.07E–01
HDL S C (mmol·L^{-1})	–0.0001	–0.0025	0.0022	9.14E–01	–0.0001	–0.0024	0.0023	9.58E–01
HDL VS C (mmol·L^{-1})	0.0001	–0.0008	0.0009	8.53E–01	0.0001	–0.0007	0.0010	7.96E–01
Total C (mmol·L^{-1})	–0.0034	–0.0337	0.0270	8.25E–01	–0.0030	–0.0337	0.0276	8.44E–01
Non-HDL C (mmol·L^{-1})	–0.0031	–0.0273	0.0212	8.01E–01	–0.0026	–0.0276	0.0223	8.32E–01
CM TG (mmol·L^{-1})	0.0012	–0.0005	0.0029	1.78E–01	0.0015	–0.0003	0.0033	9.78E–02
VLDL TG (mmol·L^{-1})	0.0026	–0.0113	0.0166	7.08E–01	0.0053	–0.0103	0.0209	4.98E–01
VLDL L1 TG (mmol·L^{-1})	0.0012	–0.0008	0.0032	2.22E–01	0.0017	–0.0005	0.0039	1.25E–01
VLDL L2 TG (mmol·L^{-1})	0.0021	–0.0019	0.0062	3.01E–01	0.0031	–0.0015	0.0076	1.83E–01
VLDL L3 TG (mmol·L^{-1})	0.0009	–0.0042	0.0060	7.19E–01	0.0019	–0.0038	0.0076	5.08E–01
VLDL M TG (mmol·L^{-1})	–0.0004	–0.0032	0.0025	8.07E–01	0.0000	–0.0031	0.0031	9.89E–01
VLDL S TG (mmol·L^{-1})	–0.0007	–0.0017	0.0004	2.01E–01	–0.0006	–0.0017	0.0004	2.42E–01
LDL TG (mmol·L^{-1})	–0.0013	–0.0033	0.0008	2.15E–01	–0.0012	–0.0033	0.0008	2.41E–01
LDL L TG (mmol·L^{-1})	–0.0008	–0.0021	0.0004	1.89E–01	–0.0008	–0.0021	0.0005	2.07E–01
LDL M TG (mmol·L^{-1})	–0.0004	–0.0012	0.0004	3.48E–01	–0.0004	–0.0012	0.0005	3.65E–01
LDL S TG (mmol·L^{-1})	0.0001	–0.0002	0.0005	3.76E–01	0.0002	–0.0002	0.0005	3.00E–01
LDL VS TG (mmol·L^{-1})	0.0001	–0.0001	0.0002	3.83E–01	0.0001	–0.0001	0.0002	2.71E–01
HDL TG (mmol·L^{-1})	–0.0006	–0.0029	0.0017	6.12E–01	–0.0005	–0.0028	0.0019	6.75E–01
HDL VL TG (mmol·L^{-1})	0.0000	–0.0002	0.0001	6.69E–01	0.0000	–0.0002	0.0001	6.30E–01
HDL L TG (mmol·L^{-1})	–0.0002	–0.0009	0.0004	5.23E–01	–0.0002	–0.0009	0.0004	4.55E–01
HDL M TG (mmol·L^{-1})	–0.0002	–0.0012	0.0007	6.09E–01	–0.0002	–0.0012	0.0008	7.02E–01

HDL S TG (mmol·L^{-1})	–0.0001	–0.0007	0.0005	7.60E–01	0.0000	–0.0006	0.0006	9.27E–01
HDL VS TG (mmol·L^{-1})	0.0000	–0.0001	0.0002	4.81E–01	0.0001	–0.0001	0.0002	2.90E–01
Total TG (mmol·L^{-1})	0.0034	–0.0141	0.0210	6.98E–01	0.0066	–0.0129	0.0261	4.98E–01
VLDL size (nm)	0.0876	–0.0400	0.2152	1.74E–01	0.1185	–0.0210	0.2580	9.45E–02
LDL size (nm)	–0.0062	–0.0166	0.0042	2.39E–01	–0.0068	–0.0172	0.0035	1.92E–01
HDL size (nm)	0.0005	–0.0098	0.0108	9.20E–01	–0.0001	–0.0104	0.0102	9.82E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 15. Associations between baseline moderate-intensity physical activity (MPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0382	–0.0642	–0.0122	4.78E–03	–0.0301	–0.0548	–0.0055	1.75E–02
VLDL L1 PN (nmol·L^{-1})	–0.1139	–0.1973	–0.0306	8.29E–03	–0.0876	–0.1657	–0.0096	2.84E–02
VLDL L2 PN (nmol·L^{-1})	–0.4173	–0.7279	–0.1067	9.37E–03	–0.3211	–0.6129	–0.0294	3.16E–02
VLDL L3 PN (nmol·L^{-1})	–0.7346	–1.4111	–0.0581	3.38E–02	–0.5477	–1.1816	0.0862	8.90E–02
VLDL M PN (nmol·L^{-1})	–0.5595	–1.3445	0.2255	1.59E–01	–0.3334	–1.0787	0.4120	3.74E–01
VLDL S PN (nmol·L^{-1})	0.1848	–0.4951	0.8647	5.88E–01	0.2700	–0.4295	0.9695	4.43E–01
LDL L PN (nmol·L^{-1})	0.3586	–2.3233	3.0405	7.90E–01	0.5238	–2.2372	3.2847	7.05E–01
LDL M PN (nmol·L^{-1})	–1.9253	–8.2246	4.3741	5.43E–01	–1.4106	–7.9024	5.0812	6.65E–01
LDL S PN (nmol·L^{-1})	–2.0133	–5.3129	1.2863	2.27E–01	–1.6625	–5.0433	1.7183	3.29E–01
LDL VS PN (nmol·1^{-1})	–1.0403	–2.6494	0.5689	2.01E–01	–0.8857	–2.5288	0.7574	2.85E–01
HDL VL PN (nmol·L^{-1})	–1.0764	–6.0858	3.9331	6.69E–01	–1.7402	–6.8905	3.4102	5.01E–01
HDL L PN (nmol·L^{-1})	–6.4509	–46.3380	33.4362	7.47E–01	–10.9988	–52.0761	30.0785	5.94E–01
HDL M PN (nmol·L^{-1})	9.9821	–34.2439	54.2081	6.53E–01	4.3478	–40.5196	49.2152	8.47E–01
HDL S PN (nmol·L^{-1})	–1.0307	–42.7645	40.7031	9.61E–01	4.5043	–38.3276	47.3362	8.34E–01
HDL VS PN (nmol·L^{-1})	–8.3757	–28.0431	11.2917	3.97E–01	–5.7361	–26.3380	14.8658	5.79E–01
CM C (mmol·L^{-1})	–0.0014	–0.0023	–0.0005	4.35E–03	–0.0011	–0.0020	–0.0002	1.50E–02
VLDL C (mmol·L^{-1})	–0.0078	–0.0193	0.0037	1.81E–01	–0.0052	–0.0162	0.0059	3.55E–01
VLDL L1 C (mmol·L^{-1})	–0.0015	–0.0026	–0.0004	9.61E–03	–0.0012	–0.0022	–0.0001	2.90E–02
VLDL L2 C (mmol·L^{-1})	–0.0035	–0.0062	–0.0008	1.26E–02	–0.0026	–0.0052	–0.0001	3.98E–02
VLDL L3 C (mmol·L^{-1})	–0.0047	–0.0098	0.0003	6.53E–02	–0.0037	–0.0086	0.0011	1.25E–01
VLDL M C (mmol·L^{-1})	–0.0023	–0.0056	0.0010	1.65E–01	–0.0015	–0.0047	0.0017	3.53E–01
VLDL S C (mmol·L^{-1})	0.0008	–0.0022	0.0038	5.90E–01	0.0010	–0.0021	0.0042	5.12E–01
LDL C (mmol·L^{-1})	–0.0120	–0.0427	0.0187	4.38E–01	–0.0096	–0.0412	0.0220	5.46E–01
LDL L C (mmol·L^{-1})	0.0007	–0.0087	0.0100	8.90E–01	0.0011	–0.0087	0.0108	8.24E–01

LDL M C (mmol·L^{-1})	–0.0049	–0.0191	0.0094	4.94E–01	–0.0038	–0.0185	0.0109	6.08E–01
LDL S C (mmol·L^{-1})	–0.0040	–0.0101	0.0022	2.00E–01	–0.0033	–0.0096	0.0030	3.00E–01
LDL VS C (mmol·L^{-1})	–0.0015	–0.0039	0.0008	1.87E–01	–0.0013	–0.0037	0.0011	2.76E–01
HDL C (mmol·L^{-1})	–0.0012	–0.0177	0.0152	8.81E–01	–0.0034	–0.0199	0.0130	6.78E–01
HDL VL C (mmol·L^{-1})	–0.0006	–0.0025	0.0014	5.63E–01	–0.0008	–0.0028	0.0011	4.02E–01
HDL L C (mmol·L^{-1})	–0.0013	–0.0098	0.0071	7.56E–01	–0.0024	–0.0111	0.0063	5.86E–01
HDL M C (mmol·L^{-1})	0.0005	–0.0055	0.0065	8.72E–01	–0.0004	–0.0064	0.0056	8.92E–01
HDL S C (mmol·L^{-1})	0.0002	–0.0027	0.0031	8.84E–01	0.0005	–0.0025	0.0035	7.41E–01
HDL VS C (mmol·L^{-1})	–0.0003	–0.0014	0.0007	5.17E–01	–0.0002	–0.0013	0.0008	6.70E–01
Total C (mmol·L^{-1})	–0.0158	–0.0577	0.0260	4.51E–01	–0.0147	–0.0572	0.0277	4.90E–01
Non-HDL C (mmol·L^{-1})	–0.0140	–0.0498	0.0217	4.35E–01	–0.0110	–0.0464	0.0245	5.38E–01
CM TG (mmol·L^{-1})	–0.0045	–0.0074	–0.0015	3.57E–03	–0.0036	–0.0064	–0.0008	1.34E–02
VLDL TG (mmol·L^{-1})	–0.0258	–0.0477	–0.0040	2.15E–02	–0.0192	–0.0397	0.0014	6.70E–02
VLDL L1 TG (mmol·L^{-1})	–0.0053	–0.0089	–0.0017	4.61E–03	–0.0042	–0.0075	–0.0008	1.68E–02
VLDL L2 TG (mmol·L^{-1})	–0.0096	–0.0167	–0.0025	8.82E–03	–0.0074	–0.0141	–0.0007	3.00E–02
VLDL L3 TG (mmol·L^{-1})	–0.0092	–0.0171	–0.0012	2.41E–02	–0.0068	–0.0142	0.0006	7.25E–02
VLDL M TG (mmol·L^{-1})	–0.0027	–0.0061	0.0006	1.07E–01	–0.0017	–0.0049	0.0015	2.81E–01
VLDL S TG (mmol·L^{-1})	–0.0001	–0.0010	0.0007	7.50E–01	0.0001	–0.0008	0.0009	8.94E–01
LDL TG (mmol·L^{-1})	–0.0005	–0.0027	0.0016	6.26E–01	–0.0003	–0.0025	0.0018	7.58E–01
LDL L TG (mmol·L^{-1})	0.0000	–0.0012	0.0012	9.75E–01	0.0001	–0.0010	0.0013	8.02E–01
LDL M TG (mmol·L^{-1})	–0.0001	–0.0011	0.0009	8.76E–01	0.0000	–0.0011	0.0010	9.32E–01
LDL S TG (mmol·L^{-1})	–0.0007	–0.0011	–0.0003	1.32E–03	–0.0006	–0.0010	–0.0002	3.01E–03
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0001	1.56E–03	–0.0003	–0.0005	–0.0001	4.76E–03
HDL TG (mmol·L^{-1})	–0.0013	–0.0035	0.0010	2.73E–01	–0.0009	–0.0032	0.0014	4.59E–01
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0002	0.0001	3.22E–01	–0.0001	–0.0003	0.0001	2.29E–01
HDL L TG (mmol·L^{-1})	0.0002	–0.0005	0.0008	6.25E–01	0.0000	–0.0006	0.0007	9.04E–01
HDL M TG (mmol·L^{-1})	–0.0004	–0.0014	0.0005	3.67E–01	–0.0002	–0.0012	0.0008	6.82E–01

HDL S TG (mmol·L^{-1})	–0.0004	–0.0011	0.0002	1.74E–01	–0.0002	–0.0009	0.0004	4.39E–01
HDL VS TG (mmol·L^{-1})	–0.0003	–0.0005	–0.0001	8.11E–03	–0.0002	–0.0004	0.0000	2.94E–02
Total TG (mmol·L^{-1})	–0.0337	–0.0610	–0.0064	1.66E–02	–0.0253	–0.0510	0.0005	5.42E–02
VLDL size (nm)	–0.3250	–0.5441	–0.1059	4.36E–03	–0.2601	–0.4685	–0.0516	1.54E–02
LDL size (nm)	0.0121	0.0016	0.0226	2.46E–02	0.0109	–0.0002	0.0219	5.40E–02
HDL size (nm)	–0.0019	–0.0131	0.0093	7.35E–01	–0.0035	–0.0152	0.0082	5.50E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity.
Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 16. Associations between change in moderate-intensity physical activity (MPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0106	–0.0157	0.0369	4.22E–01	0.0145	–0.0114	0.0405	2.67E–01
VLDL L1 PN (nmol·L^{-1})	0.0111	–0.0692	0.0914	7.82E–01	0.0254	–0.0542	0.1051	5.25E–01
VLDL L2 PN (nmol·L^{-1})	0.0350	–0.2491	0.3192	8.06E–01	0.0881	–0.1937	0.3699	5.34E–01
VLDL L3 PN (nmol·L^{-1})	–0.2306	–0.8695	0.4083	4.73E–01	–0.1223	–0.7639	0.5193	7.04E–01
VLDL M PN (nmol·L^{-1})	–0.5630	–1.3596	0.2336	1.62E–01	–0.4670	–1.2577	0.3237	2.42E–01
VLDL S PN (nmol·L^{-1})	–0.9850	–1.6634	–0.3065	5.20E–03	–0.9667	–1.6438	–0.2896	5.94E–03
LDL L PN (nmol·L^{-1})	–1.1913	–3.6290	1.2463	3.32E–01	–1.1550	–3.5964	1.2865	3.47E–01
LDL M PN (nmol·L^{-1})	2.5004	–3.3957	8.3966	3.99E–01	2.6286	–3.2097	8.4669	3.71E–01
LDL S PN (nmol·L^{-1})	1.8869	–1.1943	4.9681	2.25E–01	1.9861	–1.0460	5.0182	1.95E–01
LDL VS PN (nmol·1^{-1})	0.5127	–0.9877	2.0130	4.96E–01	0.5580	–0.9190	2.0351	4.52E–01
HDL VL PN (nmol·L^{-1})	–0.7404	–6.0243	4.5436	7.80E–01	–1.0911	–6.3625	4.1802	6.80E–01
HDL L PN (nmol·L^{-1})	2.1702	–37.5730	41.9133	9.13E–01	–0.9257	–40.6145	38.7632	9.63E–01
HDL M PN (nmol·L^{-1})	–20.0602	–57.0219	16.9015	2.82E–01	–23.0438	–60.2602	14.1726	2.20E–01
HDL S PN (nmol·L^{-1})	4.4812	–34.2539	43.2162	8.18E–01	6.1830	–32.6127	44.9787	7.51E–01
HDL VS PN (nmol·L^{-1})	10.9407	–6.8116	28.6931	2.22E–01	11.9216	–5.5045	29.3477	1.76E–01
CM C (mmol·L^{-1})	0.0001	–0.0009	0.0011	8.40E–01	0.0002	–0.0007	0.0012	6.17E–01
VLDL C (mmol·L^{-1})	–0.0138	–0.0265	–0.0011	3.41E–02	–0.0127	–0.0256	0.0002	5.35E–02
VLDL L1 C (mmol·L^{-1})	–0.0001	–0.0013	0.0010	7.95E–01	0.0000	–0.0011	0.0012	9.34E–01
VLDL L2 C (mmol·L^{-1})	–0.0005	–0.0032	0.0022	7.17E–01	–0.0001	–0.0028	0.0026	9.66E–01
VLDL L3 C (mmol·L^{-1})	–0.0031	–0.0078	0.0015	1.78E–01	–0.0027	–0.0073	0.0020	2.62E–01
VLDL M C (mmol·L^{-1})	–0.0043	–0.0077	–0.0009	1.36E–02	–0.0040	–0.0074	–0.0005	2.42E–02
VLDL S C (mmol·L^{-1})	–0.0040	–0.0071	–0.0009	1.12E–02	–0.0040	–0.0070	–0.0009	1.19E–02
LDL C (mmol·L^{-1})	0.0112	–0.0172	0.0395	4.33E–01	0.0118	–0.0163	0.0399	4.03E–01
LDL L C (mmol·L^{-1})	–0.0023	–0.0113	0.0067	6.08E–01	–0.0022	–0.0112	0.0068	6.24E–01

LDL M C (mmol·L^{-1})	0.0071	–0.0064	0.0205	2.96E–01	0.0074	–0.0060	0.0207	2.73E–01
LDL S C (mmol·L^{-1})	0.0041	–0.0017	0.0098	1.60E–01	0.0042	–0.0014	0.0098	1.35E–01
LDL VS C (mmol·L^{-1})	0.0010	–0.0011	0.0032	3.34E–01	0.0011	–0.0010	0.0032	2.95E–01
HDL C (mmol·L^{-1})	0.0006	–0.0149	0.0160	9.40E–01	–0.0010	–0.0166	0.0147	9.03E–01
HDL VL C (mmol·L^{-1})	–0.0004	–0.0024	0.0016	6.87E–01	–0.0006	–0.0026	0.0014	5.81E–01
HDL L C (mmol·L^{-1})	–0.0007	–0.0097	0.0083	8.71E–01	–0.0015	–0.0105	0.0076	7.45E–01
HDL M C (mmol·L^{-1})	–0.0010	–0.0059	0.0039	6.71E–01	–0.0015	–0.0065	0.0034	5.36E–01
HDL S C (mmol·L^{-1})	0.0010	–0.0018	0.0037	4.81E–01	0.0011	–0.0017	0.0038	4.43E–01
HDL VS C (mmol·L^{-1})	0.0006	–0.0004	0.0015	2.20E–01	0.0006	–0.0003	0.0015	1.85E–01
Total C (mmol·L^{-1})	–0.0079	–0.0445	0.0286	6.65E–01	–0.0075	–0.0440	0.0290	6.82E–01
Non-HDL C (mmol·L^{-1})	–0.0072	–0.0387	0.0243	6.49E–01	–0.0063	–0.0379	0.0253	6.89E–01
CM TG (mmol·L^{-1})	0.0012	–0.0019	0.0042	4.51E–01	0.0016	–0.0014	0.0046	2.96E–01
VLDL TG (mmol·L^{-1})	–0.0032	–0.0237	0.0173	7.54E–01	0.0004	–0.0199	0.0207	9.66E–01
VLDL L1 TG (mmol·L^{-1})	0.0008	–0.0027	0.0044	6.45E–01	0.0014	–0.0021	0.0049	4.23E–01
VLDL L2 TG (mmol·L^{-1})	0.0008	–0.0058	0.0075	8.00E–01	0.0020	–0.0046	0.0087	5.37E–01
VLDL L3 TG (mmol·L^{-1})	–0.0012	–0.0084	0.0061	7.47E–01	0.0002	–0.0070	0.0074	9.62E–01
VLDL M TG (mmol·L^{-1})	–0.0018	–0.0051	0.0014	2.60E–01	–0.0014	–0.0045	0.0018	3.92E–01
VLDL S TG (mmol·L^{-1})	–0.0012	–0.0021	–0.0003	8.12E–03	–0.0012	–0.0020	–0.0003	1.11E–02
LDL TG (mmol·L^{-1})	–0.0021	–0.0043	0.0001	6.42E–02	–0.0020	–0.0042	0.0002	7.42E–02
LDL L TG (mmol·L^{-1})	–0.0014	–0.0025	–0.0004	8.51E–03	–0.0014	–0.0024	–0.0003	1.04E–02
LDL M TG (mmol·L^{-1})	–0.0006	–0.0015	0.0003	1.75E–01	–0.0006	–0.0015	0.0003	1.85E–01
LDL S TG (mmol·L^{-1})	0.0001	–0.0004	0.0006	6.29E–01	0.0001	–0.0003	0.0006	5.40E–01
LDL VS TG (mmol·L^{-1})	0.0000	–0.0002	0.0003	7.42E–01	0.0001	–0.0002	0.0003	5.97E–01
HDL TG (mmol·L^{-1})	–0.0021	–0.0048	0.0005	1.12E–01	–0.0020	–0.0047	0.0006	1.31E–01
HDL VL TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	8.13E–02	–0.0002	–0.0004	0.0000	7.83E–02
HDL L TG (mmol·L^{-1})	–0.0008	–0.0015	0.0000	4.85E–02	–0.0008	–0.0016	0.0000	3.81E–02
HDL M TG (mmol·L^{-1})	–0.0007	–0.0018	0.0003	1.75E–01	–0.0007	–0.0017	0.0004	2.15E–01

HDL S TG (mmol·L^{-1})	–0.0003	–0.0010	0.0004	4.59E–01	–0.0002	–0.0008	0.0005	6.21E–01
HDL VS TG (mmol·L^{-1})	0.0000	–0.0002	0.0002	7.61E–01	0.0001	–0.0001	0.0003	4.95E–01
Total TG (mmol·L^{-1})	–0.0044	–0.0315	0.0227	7.47E–01	–0.0001	–0.0269	0.0267	9.94E–01
VLDL size (nm)	0.0757	–0.1280	0.2793	4.60E–01	0.1134	–0.0892	0.3161	2.67E–01
LDL size (nm)	–0.0138	–0.0228	–0.0048	3.20E–03	–0.0144	–0.0233	–0.0056	1.92E–03
HDL size (nm)	–0.0038	–0.0154	0.0079	5.21E–01	–0.0048	–0.0165	0.0069	4.14E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 17. Associations between baseline light-intensity physical activity (LPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0185	–0.0431	0.0062	1.40E–01	–0.0172	–0.0415	0.0070	1.60E–01
VLDL L1 PN (nmol·L^{-1})	–0.0453	–0.1214	0.0309	2.39E–01	–0.0419	–0.1143	0.0305	2.51E–01
VLDL L2 PN (nmol·L^{-1})	–0.1762	–0.4437	0.0914	1.93E–01	–0.1652	–0.4170	0.0866	1.94E–01
VLDL L3 PN (nmol·L^{-1})	–0.0125	–0.5968	0.5717	9.66E–01	0.0079	–0.5278	0.5436	9.76E–01
VLDL M PN (nmol·L^{-1})	0.0810	–0.6827	0.8446	8.33E–01	0.1006	–0.6010	0.8022	7.75E–01
VLDL S PN (nmol·L^{-1})	0.5186	–0.3181	1.3553	2.20E–01	0.5320	–0.3045	1.3686	2.08E–01
LDL L PN (nmol·L^{-1})	2.5262	0.0932	4.9591	4.21E–02	2.5597	0.1172	5.0022	4.03E–02
LDL M PN (nmol·L^{-1})	5.4295	0.1334	10.7256	4.47E–02	5.5798	0.3067	10.8529	3.85E–02
LDL S PN (nmol·L^{-1})	2.4162	–0.5966	5.4289	1.14E–01	2.5299	–0.4694	5.5293	9.66E–02
LDL VS PN (nmol·1^{-1})	0.7679	–0.6816	2.2175	2.93E–01	0.8123	–0.6372	2.2618	2.66E–01
HDL VL PN (nmol·L^{-1})	–7.5808	–12.0786	–3.0830	1.34E–03	–7.7217	–12.0542	–3.3893	7.41E–04
HDL L PN (nmol·L^{-1})	–50.7116	–83.0841	–18.3390	2.71E–03	–51.6776	–82.9954	–20.3598	1.66E–03
HDL M PN (nmol·L^{-1})	–22.6285	–58.4923	13.2353	2.11E–01	–23.6760	–58.9766	11.6246	1.85E–01
HDL S PN (nmol·L^{-1})	34.9046	–3.9806	73.7898	7.75E–02	36.1746	–2.2388	74.5881	6.44E–02
HDL VS PN (nmol·L^{-1})	16.0605	–1.1641	33.2851	6.70E–02	16.7175	–0.4156	33.8506	5.56E–02
CM C (mmol·L^{-1})	–0.0007	–0.0015	0.0002	1.44E–01	–0.0006	–0.0015	0.0002	1.55E–01
VLDL C (mmol·L^{-1})	0.0016	–0.0118	0.0150	8.10E–01	0.0020	–0.0109	0.0149	7.59E–01
VLDL L1 C (mmol·L^{-1})	–0.0003	–0.0013	0.0007	5.59E–01	–0.0003	–0.0012	0.0007	5.85E–01
VLDL L2 C (mmol·L^{-1})	–0.0010	–0.0034	0.0014	4.06E–01	–0.0009	–0.0032	0.0013	4.13E–01
VLDL L3 C (mmol·L^{-1})	0.0018	–0.0023	0.0059	3.92E–01	0.0019	–0.0020	0.0058	3.29E–01
VLDL M C (mmol·L^{-1})	–0.0011	–0.0049	0.0028	5.84E–01	–0.0010	–0.0047	0.0027	5.91E–01
VLDL S C (mmol·L^{-1})	0.0017	–0.0021	0.0054	3.72E–01	0.0017	–0.0021	0.0055	3.66E–01
LDL C (mmol·L^{-1})	0.0212	–0.0048	0.0471	1.08E–01	0.0218	–0.0041	0.0477	9.69E–02
LDL L C (mmol·L^{-1})	0.0085	0.0002	0.0168	4.58E–02	0.0086	0.0002	0.0170	4.55E–02

LDL M C (mmol·L^{-1})	0.0127	0.0009	0.0246	3.52E–02	0.0131	0.0013	0.0248	3.05E–02
LDL S C (mmol·L^{-1})	0.0041	–0.0015	0.0096	1.45E–01	0.0043	–0.0012	0.0098	1.24E–01
LDL VS C (mmol·L^{-1})	0.0012	–0.0009	0.0033	2.52E–01	0.0013	–0.0008	0.0034	2.27E–01
HDL C (mmol·L^{-1})	–0.0152	–0.0277	–0.0027	1.79E–02	–0.0155	–0.0274	–0.0036	1.19E–02
HDL VL C (mmol·L^{-1})	–0.0027	–0.0045	–0.0009	3.19E–03	–0.0028	–0.0045	–0.0011	1.85E–03
HDL L C (mmol·L^{-1})	–0.0109	–0.0185	–0.0034	5.22E–03	–0.0112	–0.0184	–0.0039	3.30E–03
HDL M C (mmol·L^{-1})	–0.0037	–0.0081	0.0008	1.04E–01	–0.0038	–0.0080	0.0004	7.61E–02
HDL S C (mmol·L^{-1})	0.0024	–0.0002	0.0051	7.32E–02	0.0025	–0.0002	0.0051	6.51E–02
HDL VS C (mmol·L^{-1})	0.0007	–0.0002	0.0016	1.06E–01	0.0008	–0.0001	0.0017	9.79E–02
Total C (mmol·L^{-1})	0.0096	–0.0258	0.0449	5.90E–01	0.0098	–0.0255	0.0452	5.79E–01
Non-HDL C (mmol·L^{-1})	0.0233	–0.0097	0.0562	1.63E–01	0.0241	–0.0085	0.0568	1.45E–01
CM TG (mmol·L^{-1})	–0.0020	–0.0048	0.0008	1.57E–01	–0.0019	–0.0046	0.0009	1.82E–01
VLDL TG (mmol·L^{-1})	–0.0052	–0.0243	0.0138	5.85E–01	–0.0045	–0.0223	0.0133	6.14E–01
VLDL L1 TG (mmol·L^{-1})	–0.0022	–0.0054	0.0010	1.69E–01	–0.0021	–0.0052	0.0010	1.84E–01
VLDL L2 TG (mmol·L^{-1})	–0.0034	–0.0095	0.0027	2.68E–01	–0.0031	–0.0089	0.0026	2.82E–01
VLDL L3 TG (mmol·L^{-1})	–0.0014	–0.0081	0.0053	6.85E–01	–0.0011	–0.0073	0.0051	7.20E–01
VLDL M TG (mmol·L^{-1})	0.0006	–0.0026	0.0037	7.23E–01	0.0007	–0.0023	0.0036	6.56E–01
VLDL S TG (mmol·L^{-1})	0.0007	–0.0004	0.0017	2.02E–01	0.0007	–0.0003	0.0017	1.77E–01
LDL TG (mmol·L^{-1})	0.0010	–0.0015	0.0035	4.36E–01	0.0010	–0.0015	0.0035	4.26E–01
LDL L TG (mmol·L^{-1})	0.0007	–0.0005	0.0019	2.65E–01	0.0007	–0.0005	0.0020	2.53E–01
LDL M TG (mmol·L^{-1})	0.0003	–0.0007	0.0014	5.48E–01	0.0003	–0.0007	0.0014	5.42E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0008	0.0000	5.07E–02	–0.0004	–0.0008	0.0000	5.89E–02
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	8.27E–02	–0.0002	–0.0004	0.0000	9.61E–02
HDL TG (mmol·L^{-1})	–0.0009	–0.0036	0.0018	5.09E–01	–0.0009	–0.0036	0.0019	5.30E–01
HDL VL TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	1.85E–02	–0.0002	–0.0004	0.0000	1.61E–02
HDL L TG (mmol·L^{-1})	–0.0003	–0.0010	0.0004	3.80E–01	–0.0003	–0.0010	0.0003	3.41E–01
HDL M TG (mmol·L^{-1})	0.0000	–0.0012	0.0012	9.99E–01	0.0000	–0.0011	0.0012	9.61E–01

HDL S TG (mmol·L^{-1})	0.0002	–0.0005	0.0009	6.41E–01	0.0002	–0.0005	0.0009	5.77E–01
HDL VS TG (mmol·L^{-1})	0.0000	–0.0002	0.0001	6.11E–01	0.0000	–0.0002	0.0001	6.56E–01
Total TG (mmol·L^{-1})	–0.0093	–0.0342	0.0156	4.57E–01	–0.0083	–0.0319	0.0152	4.81E–01
VLDL size (nm)	–0.1168	–0.2955	0.0620	1.96E–01	–0.1087	–0.2802	0.0627	2.09E–01
LDL size (nm)	0.0015	–0.0100	0.0130	7.94E–01	0.0013	–0.0103	0.0129	8.25E–01
HDL size (nm)	–0.0158	–0.0261	–0.0055	3.34E–03	–0.0162	–0.0261	–0.0064	1.72E–03

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity.
Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 18. Associations between change in light-intensity physical activity (LPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0154	–0.0195	0.0503	3.82E–01	0.0211	–0.0135	0.0556	2.27E–01
VLDL L1 PN (nmol·L^{-1})	0.0163	–0.0842	0.1169	7.46E–01	0.0364	–0.0631	0.1360	4.66E–01
VLDL L2 PN (nmol·L^{-1})	0.0063	–0.3423	0.3549	9.71E–01	0.0813	–0.2627	0.4254	6.38E–01
VLDL L3 PN (nmol·L^{-1})	–0.2353	–0.9772	0.5067	5.28E–01	–0.0865	–0.8080	0.6350	8.11E–01
VLDL M PN (nmol·L^{-1})	–0.4388	–1.4030	0.5254	3.66E–01	–0.2843	–1.1942	0.6256	5.34E–01
VLDL S PN (nmol·L^{-1})	–0.3548	–1.1115	0.4019	3.52E–01	–0.3149	–1.0358	0.4060	3.85E–01
LDL L PN (nmol·L^{-1})	–0.5804	–2.9999	1.8390	6.33E–01	–0.4907	–2.8443	1.8628	6.78E–01
LDL M PN (nmol·L^{-1})	0.0889	–5.4470	5.6248	9.74E–01	0.4230	–5.1360	5.9820	8.79E–01
LDL S PN (nmol·L^{-1})	0.1158	–3.0951	3.3267	9.43E–01	0.3603	–2.8580	3.5785	8.23E–01
LDL VS PN (nmol·1^{-1})	0.0616	–1.4823	1.6055	9.37E–01	0.1714	–1.3817	1.7246	8.26E–01
HDL VL PN (nmol·L^{-1})	1.8640	–3.4811	7.2091	4.88E–01	1.3078	–3.8547	6.4703	6.14E–01
HDL L PN (nmol·L^{-1})	12.2894	–29.2411	53.8199	5.56E–01	7.8932	–32.6521	48.4384	6.98E–01
HDL M PN (nmol·L^{-1})	12.5207	–25.9716	51.0130	5.17E–01	7.4208	–31.4532	46.2948	7.04E–01
HDL S PN (nmol·L^{-1})	1.4009	–36.7522	39.5539	9.42E–01	4.5969	–33.2153	42.4090	8.08E–01
HDL VS PN (nmol·L^{-1})	–4.2003	–23.8743	15.4736	6.71E–01	–2.2451	–21.5280	17.0378	8.16E–01
CM C (mmol·L^{-1})	0.0004	–0.0009	0.0016	5.61E–01	0.0006	–0.0006	0.0018	3.46E–01
VLDL C (mmol·L^{-1})	0.0007	–0.0142	0.0156	9.22E–01	0.0024	–0.0121	0.0169	7.41E–01
VLDL L1 C (mmol·L^{-1})	0.0001	–0.0013	0.0014	9.25E–01	0.0003	–0.0010	0.0016	6.18E–01
VLDL L2 C (mmol·L^{-1})	–0.0001	–0.0033	0.0031	9.70E–01	0.0006	–0.0025	0.0037	7.12E–01
VLDL L3 C (mmol·L^{-1})	–0.0020	–0.0074	0.0033	4.53E–01	–0.0013	–0.0066	0.0041	6.39E–01
VLDL M C (mmol·L^{-1})	0.0008	–0.0038	0.0053	7.30E–01	0.0013	–0.0031	0.0057	5.51E–01
VLDL S C (mmol·L^{-1})	–0.0009	–0.0041	0.0023	5.63E–01	–0.0008	–0.0039	0.0022	5.80E–01
LDL C (mmol·L^{-1})	0.0016	–0.0243	0.0276	9.00E–01	0.0032	–0.0227	0.0291	8.05E–01
LDL L C (mmol·L^{-1})	–0.0030	–0.0119	0.0059	4.99E–01	–0.0028	–0.0114	0.0058	5.18E–01

LDL M C (mmol·L^{-1})	–0.0004	–0.0127	0.0119	9.44E–01	0.0003	–0.0119	0.0126	9.59E–01
LDL S C (mmol·L^{-1})	0.0004	–0.0053	0.0061	8.93E–01	0.0009	–0.0048	0.0065	7.64E–01
LDL VS C (mmol·L^{-1})	0.0001	–0.0020	0.0022	8.95E–01	0.0003	–0.0018	0.0024	7.72E–01
HDL C (mmol·L^{-1})	0.0063	–0.0086	0.0212	3.99E–01	0.0041	–0.0106	0.0188	5.80E–01
HDL VL C (mmol·L^{-1})	0.0009	–0.0010	0.0029	3.38E–01	0.0007	–0.0012	0.0026	4.50E–01
HDL L C (mmol·L^{-1})	0.0028	–0.0061	0.0117	5.29E–01	0.0018	–0.0069	0.0104	6.80E–01
HDL M C (mmol·L^{-1})	0.0030	–0.0016	0.0075	1.97E–01	0.0022	–0.0024	0.0067	3.44E–01
HDL S C (mmol·L^{-1})	–0.0002	–0.0028	0.0023	8.57E–01	–0.0001	–0.0026	0.0025	9.60E–01
HDL VS C (mmol·L^{-1})	–0.0002	–0.0012	0.0007	6.39E–01	–0.0002	–0.0011	0.0008	7.53E–01
Total C (mmol·L^{-1})	0.0084	–0.0259	0.0427	6.25E–01	0.0095	–0.0247	0.0437	5.79E–01
Non-HDL C (mmol·L^{-1})	0.0044	–0.0259	0.0348	7.70E–01	0.0064	–0.0239	0.0366	6.75E–01
CM TG (mmol·L^{-1})	0.0021	–0.0020	0.0061	3.09E–01	0.0027	–0.0013	0.0067	1.79E–01
VLDL TG (mmol·L^{-1})	–0.0021	–0.0264	0.0222	8.63E–01	0.0030	–0.0207	0.0267	8.00E–01
VLDL L1 TG (mmol·L^{-1})	0.0014	–0.0030	0.0058	5.33E–01	0.0022	–0.0021	0.0066	3.11E–01
VLDL L2 TG (mmol·L^{-1})	0.0008	–0.0072	0.0088	8.47E–01	0.0025	–0.0054	0.0104	5.31E–01
VLDL L3 TG (mmol·L^{-1})	–0.0016	–0.0101	0.0069	7.03E–01	0.0003	–0.0080	0.0085	9.52E–01
VLDL M TG (mmol·L^{-1})	–0.0021	–0.0059	0.0017	2.78E–01	–0.0014	–0.0050	0.0023	4.54E–01
VLDL S TG (mmol·L^{-1})	–0.0006	–0.0016	0.0005	2.84E–01	–0.0005	–0.0015	0.0006	3.71E–01
LDL TG (mmol·L^{-1})	0.0008	–0.0023	0.0038	6.24E–01	0.0009	–0.0022	0.0040	5.74E–01
LDL L TG (mmol·L^{-1})	–0.0002	–0.0014	0.0011	7.93E–01	–0.0001	–0.0013	0.0012	8.82E–01
LDL M TG (mmol·L^{-1})	0.0004	–0.0008	0.0015	5.36E–01	0.0004	–0.0008	0.0016	5.13E–01
LDL S TG (mmol·L^{-1})	0.0004	–0.0003	0.0010	2.46E–01	0.0004	–0.0002	0.0010	1.82E–01
LDL VS TG (mmol·L^{-1})	0.0002	–0.0002	0.0005	3.32E–01	0.0002	–0.0001	0.0005	2.22E–01
HDL TG (mmol·L^{-1})	0.0005	–0.0027	0.0038	7.42E–01	0.0008	–0.0024	0.0039	6.36E–01
HDL VL TG (mmol·L^{-1})	0.0001	–0.0001	0.0003	3.41E–01	0.0001	–0.0001	0.0003	4.03E–01
HDL L TG (mmol·L^{-1})	0.0003	–0.0005	0.0011	5.12E–01	0.0002	–0.0007	0.0010	6.67E–01
HDL M TG (mmol·L^{-1})	0.0001	–0.0012	0.0015	8.65E–01	0.0002	–0.0010	0.0015	7.07E–01

HDL S TG (mmol·L^{-1})	0.0001	–0.0008	0.0010	8.26E–01	0.0002	–0.0006	0.0010	5.89E–01
HDL VS TG (mmol·L^{-1})	0.0001	–0.0002	0.0003	6.62E–01	0.0001	–0.0001	0.0003	3.92E–01
Total TG (mmol·L^{-1})	0.0016	–0.0312	0.0343	9.25E–01	0.0077	–0.0241	0.0394	6.31E–01
VLDL size (nm)	0.0503	–0.2013	0.3019	6.90E–01	0.1026	–0.1473	0.3524	4.14E–01
LDL size (nm)	–0.0041	–0.0162	0.0081	5.04E–01	–0.0053	–0.0171	0.0065	3.73E–01
HDL size (nm)	0.0039	–0.0081	0.0158	5.21E–01	0.0024	–0.0092	0.0140	6.83E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 19. Associations between baseline sedentary time and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0435	0.0141	0.0728	4.45E–03	0.0346	0.0074	0.0617	1.35E–02
VLDL L1 PN (nmol·L^{-1})	0.1218	0.0304	0.2132	9.92E–03	0.0926	0.0097	0.1754	2.92E–02
VLDL L2 PN (nmol·L^{-1})	0.4531	0.1276	0.7787	7.23E–03	0.3476	0.0522	0.6430	2.19E–02
VLDL L3 PN (nmol·L^{-1})	0.5786	–0.1062	1.2634	9.61E–02	0.3684	–0.2470	0.9838	2.36E–01
VLDL M PN (nmol·L^{-1})	0.4283	–0.4507	1.3074	3.33E–01	0.1824	–0.6441	1.0088	6.60E–01
VLDL S PN (nmol·L^{-1})	–0.3936	–1.2882	0.5011	3.82E–01	–0.4876	–1.4312	0.4560	3.05E–01
LDL L PN (nmol·L^{-1})	–1.6423	–4.7158	1.4313	2.89E–01	–1.8430	–5.0281	1.3421	2.51E–01
LDL M PN (nmol·L^{-1})	–1.9206	–8.9266	5.0854	5.85E–01	–2.5777	–9.6757	4.5202	4.70E–01
LDL S PN (nmol·L^{-1})	–0.2138	–4.0090	3.5815	9.11E–01	–0.6610	–4.4907	3.1687	7.31E–01
LDL VS PN (nmol·1^{-1})	0.2335	–1.6192	2.0863	8.02E–01	0.0429	–1.8255	1.9114	9.63E–01
HDL VL PN (nmol·L^{-1})	5.2069	0.0091	10.4048	4.96E–02	6.0354	0.8816	11.1893	2.25E–02
HDL L PN (nmol·L^{-1})	32.2079	–6.2829	70.6988	9.93E–02	37.9489	–0.1736	76.0714	5.10E–02
HDL M PN (nmol·L^{-1})	8.5382	–35.2908	52.3673	6.98E–01	15.1323	–28.6523	58.9169	4.92E–01
HDL S PN (nmol·L^{-1})	–19.3194	–60.5987	21.9599	3.53E–01	–25.7449	–66.8657	15.3760	2.15E–01
HDL VS PN (nmol·L^{-1})	–5.6793	–25.8369	14.4783	5.75E–01	–8.8155	–29.5220	11.8910	3.97E–01
CM C (mmol·L^{-1})	0.0015	0.0005	0.0026	5.03E–03	0.0012	0.0003	0.0022	1.44E–02
VLDL C (mmol·L^{-1})	0.0068	–0.0076	0.0213	3.48E–01	0.0038	–0.0102	0.0178	5.87E–01
VLDL L1 C (mmol·L^{-1})	0.0014	0.0002	0.0026	2.46E–02	0.0010	–0.0001	0.0021	6.35E–02
VLDL L2 C (mmol·L^{-1})	0.0034	0.0005	0.0063	2.12E–02	0.0025	–0.0001	0.0051	5.91E–02
VLDL L3 C (mmol·L^{-1})	0.0022	–0.0029	0.0073	3.89E–01	0.0011	–0.0037	0.0058	6.56E–01
VLDL M C (mmol·L^{-1})	0.0034	–0.0007	0.0076	1.06E–01	0.0026	–0.0015	0.0067	2.13E–01
VLDL S C (mmol·L^{-1})	–0.0012	–0.0052	0.0027	5.35E–01	–0.0015	–0.0058	0.0028	4.90E–01
LDL C (mmol·L^{-1})	–0.0044	–0.0389	0.0300	7.98E–01	–0.0074	–0.0423	0.0275	6.72E–01
LDL L C (mmol·L^{-1})	–0.0057	–0.0160	0.0046	2.72E–01	–0.0062	–0.0170	0.0045	2.51E–01

LDL M C (mmol·L^{-1})	–0.0050	–0.0206	0.0107	5.28E–01	–0.0064	–0.0223	0.0095	4.24E–01
LDL S C (mmol·L^{-1})	0.0000	–0.0070	0.0071	9.89E–01	–0.0008	–0.0079	0.0063	8.22E–01
LDL VS C (mmol·L^{-1})	0.0003	–0.0024	0.0030	8.30E–01	0.0000	–0.0027	0.0027	9.99E–01
HDL C (mmol·L^{-1})	0.0094	–0.0066	0.0254	2.46E–01	0.0120	–0.0034	0.0275	1.25E–01
HDL VL C (mmol·L^{-1})	0.0019	–0.0001	0.0040	6.11E–02	0.0023	0.0003	0.0043	2.75E–02
HDL L C (mmol·L^{-1})	0.0070	–0.0017	0.0156	1.11E–01	0.0083	–0.0003	0.0169	5.71E–02
HDL M C (mmol·L^{-1})	0.0018	–0.0039	0.0075	5.28E–01	0.0029	–0.0027	0.0084	3.02E–01
HDL S C (mmol·L^{-1})	–0.0016	–0.0046	0.0014	2.82E–01	–0.0019	–0.0049	0.0010	1.98E–01
HDL VS C (mmol·L^{-1})	–0.0003	–0.0014	0.0008	5.95E–01	–0.0004	–0.0016	0.0007	4.71E–01
Total C (mmol·L^{-1})	0.0068	–0.0405	0.0542	7.74E–01	0.0054	–0.0425	0.0533	8.22E–01
Non-HDL C (mmol·L^{-1})	–0.0019	–0.0440	0.0402	9.28E–01	–0.0057	–0.0474	0.0359	7.84E–01
CM TG (mmol·L^{-1})	0.0049	0.0016	0.0083	4.21E–03	0.0039	0.0009	0.0070	1.29E–02
VLDL TG (mmol·L^{-1})	0.0240	0.0015	0.0464	3.70E–02	0.0166	–0.0038	0.0371	1.08E–01
VLDL L1 TG (mmol·L^{-1})	0.0057	0.0018	0.0096	4.81E–03	0.0045	0.0009	0.0080	1.46E–02
VLDL L2 TG (mmol·L^{-1})	0.0099	0.0025	0.0173	9.50E–03	0.0075	0.0008	0.0142	2.90E–02
VLDL L3 TG (mmol·L^{-1})	0.0081	0.0002	0.0160	4.51E–02	0.0055	–0.0017	0.0127	1.29E–01
VLDL M TG (mmol·L^{-1})	0.0018	–0.0017	0.0053	3.02E–01	0.0007	–0.0025	0.0040	6.60E–01
VLDL S TG (mmol·L^{-1})	–0.0003	–0.0014	0.0008	5.55E–01	–0.0005	–0.0017	0.0006	3.34E–01
LDL TG (mmol·L^{-1})	–0.0001	–0.0031	0.0028	9.24E–01	–0.0004	–0.0032	0.0025	8.08E–01
LDL L TG (mmol·L^{-1})	–0.0004	–0.0019	0.0011	5.87E–01	–0.0005	–0.0020	0.0009	4.65E–01
LDL M TG (mmol·L^{-1})	–0.0001	–0.0013	0.0012	8.95E–01	–0.0001	–0.0014	0.0011	8.46E–01
LDL S TG (mmol·L^{-1})	0.0008	0.0003	0.0012	1.45E–03	0.0007	0.0002	0.0011	2.75E–03
LDL VS TG (mmol·L^{-1})	0.0004	0.0002	0.0007	1.69E–03	0.0003	0.0001	0.0006	4.14E–03
HDL TG (mmol·L^{-1})	0.0017	–0.0012	0.0046	2.54E–01	0.0013	–0.0017	0.0043	4.02E–01
HDL VL TG (mmol·L^{-1})	0.0002	0.0000	0.0004	4.71E–02	0.0002	0.0000	0.0004	2.76E–02
HDL L TG (mmol·L^{-1})	0.0000	–0.0007	0.0008	9.25E–01	0.0002	–0.0006	0.0009	6.65E–01
HDL M TG (mmol·L^{-1})	0.0004	–0.0008	0.0017	4.96E–01	0.0002	–0.0011	0.0014	7.86E–01

HDL S TG (mmol·L^{-1})	0.0003	–0.0005	0.0011	4.68E–01	0.0001	–0.0007	0.0008	8.59E–01
HDL VS TG (mmol·L^{-1})	0.0002	0.0000	0.0005	2.14E–02	0.0002	0.0000	0.0004	6.22E–02
Total TG (mmol·L^{-1})	0.0332	0.0040	0.0624	2.68E–02	0.0240	–0.0029	0.0508	7.89E–02
VLDL size (nm)	0.3324	0.1090	0.5558	4.25E–03	0.2612	0.0569	0.4656	1.32E–02
LDL size (nm)	–0.0087	–0.0217	0.0043	1.84E–01	–0.0073	–0.0211	0.0065	2.92E–01
HDL size (nm)	0.0100	–0.0013	0.0214	8.24E–02	0.0121	0.0009	0.0233	3.53E–02

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity.
Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Supplementary table 20. Associations between change in sedentary time and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0208	–0.0572	0.0156	2.57E–01	–0.0280	–0.0650	0.0089	1.34E–01
VLDL L1 PN (nmol·L^{-1})	–0.0278	–0.1329	0.0773	5.98E–01	–0.0533	–0.1609	0.0544	3.26E–01
VLDL L2 PN (nmol·L^{-1})	–0.0632	–0.4262	0.2999	7.29E–01	–0.1581	–0.5306	0.2144	3.99E–01
VLDL L3 PN (nmol·L^{-1})	0.2758	–0.5123	1.0640	4.86E–01	0.0910	–0.7109	0.8930	8.21E–01
VLDL M PN (nmol·L^{-1})	0.5925	–0.4723	1.6572	2.70E–01	0.4134	–0.6324	1.4593	4.32E–01
VLDL S PN (nmol·L^{-1})	0.8479	0.0131	1.6826	4.66E–02	0.8078	–0.0024	1.6181	5.07E–02
LDL L PN (nmol·L^{-1})	1.1970	–1.3815	3.7754	3.56E–01	1.1072	–1.4480	3.6623	3.89E–01
LDL M PN (nmol·L^{-1})	–1.1072	–7.2414	5.0271	7.19E–01	–1.4464	–7.6239	4.7311	6.41E–01
LDL S PN (nmol·L^{-1})	–0.8629	–4.4405	2.7146	6.31E–01	–1.1161	–4.7132	2.4809	5.37E–01
LDL VS PN (nmol·1^{-1})	–0.2108	–1.9278	1.5061	8.07E–01	–0.3254	–2.0565	1.4057	7.08E–01
HDL VL PN (nmol·L^{-1})	–1.2934	–7.6965	5.1097	6.87E–01	–0.6608	–6.8660	5.5444	8.32E–01
HDL L PN (nmol·L^{-1})	–12.8189	–60.9713	35.3335	5.96E–01	–7.5945	–54.1897	39.0006	7.45E–01
HDL M PN (nmol·L^{-1})	0.6975	–41.5661	42.9611	9.74E–01	6.7164	–35.3489	48.7818	7.50E–01
HDL S PN (nmol·L^{-1})	–2.0608	–41.7468	37.6252	9.18E–01	–5.6777	–45.8857	34.5303	7.78E–01
HDL VS PN (nmol·L^{-1})	–1.6689	–22.5853	19.2476	8.74E–01	–3.9137	–24.8108	16.9835	7.09E–01
CM C (mmol·L^{-1})	–0.0004	–0.0017	0.0008	4.86E–01	–0.0007	–0.0020	0.0006	2.76E–01
VLDL C (mmol·L^{-1})	0.0069	–0.0090	0.0228	3.87E–01	0.0050	–0.0109	0.0209	5.30E–01
VLDL L1 C (mmol·L^{-1})	0.0000	–0.0015	0.0014	9.52E–01	–0.0004	–0.0018	0.0011	6.03E–01
VLDL L2 C (mmol·L^{-1})	0.0000	–0.0034	0.0035	9.82E–01	–0.0008	–0.0042	0.0027	6.66E–01
VLDL L3 C (mmol·L^{-1})	0.0034	–0.0022	0.0090	2.24E–01	0.0025	–0.0032	0.0083	3.85E–01
VLDL M C (mmol·L^{-1})	0.0018	–0.0027	0.0064	4.18E–01	0.0012	–0.0032	0.0057	5.82E–01
VLDL S C (mmol·L^{-1})	0.0030	–0.0007	0.0067	1.14E–01	0.0029	–0.0007	0.0065	1.12E–01
LDL C (mmol·L^{-1})	–0.0056	–0.0346	0.0233	6.98E–01	–0.0072	–0.0364	0.0219	6.21E–01
LDL L C (mmol·L^{-1})	0.0041	–0.0055	0.0137	3.96E–01	0.0038	–0.0056	0.0133	4.17E–01

LDL M C (mmol·L^{-1})	–0.0025	–0.0162	0.0112	7.14E–01	–0.0033	–0.0171	0.0105	6.34E–01
LDL S C (mmol·L^{-1})	–0.0020	–0.0084	0.0043	5.28E–01	–0.0025	–0.0089	0.0039	4.36E–01
LDL VS C (mmol·L^{-1})	–0.0005	–0.0028	0.0019	6.88E–01	–0.0006	–0.0030	0.0017	5.86E–01
HDL C (mmol·L^{-1})	–0.0060	–0.0238	0.0118	5.04E–01	–0.0033	–0.0208	0.0141	7.03E–01
HDL VL C (mmol·L^{-1})	–0.0006	–0.0030	0.0018	6.03E–01	–0.0004	–0.0027	0.0019	7.57E–01
HDL L C (mmol·L^{-1})	–0.0025	–0.0133	0.0084	6.50E–01	–0.0012	–0.0118	0.0093	8.13E–01
HDL M C (mmol·L^{-1})	–0.0016	–0.0067	0.0035	5.24E–01	–0.0007	–0.0057	0.0044	7.89E–01
HDL S C (mmol·L^{-1})	–0.0001	–0.0029	0.0027	9.20E–01	–0.0003	–0.0031	0.0025	8.16E–01
HDL VS C (mmol·L^{-1})	–0.0001	–0.0012	0.0010	9.08E–01	–0.0002	–0.0012	0.0009	7.82E–01
Total C (mmol·L^{-1})	–0.0015	–0.0415	0.0385	9.39E–01	–0.0027	–0.0430	0.0376	8.95E–01
Non-HDL C (mmol·L^{-1})	0.0011	–0.0322	0.0344	9.48E–01	–0.0008	–0.0347	0.0330	9.61E–01
CM TG (mmol·L^{-1})	–0.0026	–0.0068	0.0016	2.27E–01	–0.0034	–0.0076	0.0009	1.18E–01
VLDL TG (mmol·L^{-1})	0.0015	–0.0239	0.0269	9.07E–01	–0.0049	–0.0308	0.0210	7.05E–01
VLDL L1 TG (mmol·L^{-1})	–0.0019	–0.0065	0.0027	4.02E–01	–0.0030	–0.0077	0.0017	2.05E–01
VLDL L2 TG (mmol·L^{-1})	–0.0019	–0.0103	0.0065	6.51E–01	–0.0041	–0.0127	0.0046	3.49E–01
VLDL L3 TG (mmol·L^{-1})	0.0012	–0.0077	0.0101	7.82E–01	–0.0011	–0.0102	0.0080	8.06E–01
VLDL M TG (mmol·L^{-1})	0.0024	–0.0017	0.0065	2.42E–01	0.0015	–0.0025	0.0056	4.48E–01
VLDL S TG (mmol·L^{-1})	0.0012	0.0000	0.0023	4.60E–02	0.0011	–0.0001	0.0022	6.81E–02
LDL TG (mmol·L^{-1})	0.0007	–0.0026	0.0041	6.63E–01	0.0006	–0.0028	0.0040	7.23E–01
LDL L TG (mmol·L^{-1})	0.0010	–0.0004	0.0024	1.56E–01	0.0009	–0.0005	0.0024	1.89E–01
LDL M TG (mmol·L^{-1})	0.0001	–0.0012	0.0014	8.60E–01	0.0001	–0.0013	0.0014	8.95E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0010	0.0003	2.44E–01	–0.0005	–0.0011	0.0002	1.80E–01
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0005	0.0002	3.30E–01	–0.0002	–0.0006	0.0001	2.15E–01
HDL TG (mmol·L^{-1})	0.0006	–0.0030	0.0042	7.39E–01	0.0004	–0.0032	0.0039	8.42E–01
HDL VL TG (mmol·L^{-1})	0.0000	–0.0002	0.0002	9.96E–01	0.0000	–0.0002	0.0003	9.26E–01
HDL L TG (mmol·L^{-1})	0.0002	–0.0008	0.0011	7.15E–01	0.0003	–0.0007	0.0012	5.69E–01
HDL M TG (mmol·L^{-1})	0.0003	–0.0012	0.0017	7.12E–01	0.0001	–0.0013	0.0015	8.64E–01

HDL S TG (mmol·L^{-1})	0.0001	–0.0009	0.0010	9.12E–01	–0.0001	–0.0010	0.0008	8.17E–01
HDL VS TG (mmol·L^{-1})	–0.0001	–0.0003	0.0002	5.78E–01	–0.0001	–0.0004	0.0001	3.00E–01
Total TG (mmol·L^{-1})	–0.0013	–0.0358	0.0331	9.39E–01	–0.0090	–0.0438	0.0258	6.07E–01
VLDL size (nm)	–0.1062	–0.3673	0.1549	4.19E–01	–0.1738	–0.4433	0.0956	2.02E–01
LDL size (nm)	0.0107	–0.0029	0.0243	1.20E–01	0.0122	–0.0011	0.0254	7.17E–02
HDL size (nm)	–0.0017	–0.0154	0.0119	8.00E–01	0.0000	–0.0131	0.0132	9.95E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Paper I

Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study.

Abstract

Background: Physical activity is favourably associated with certain markers of lipid metabolism. The relationship of physical activity with lipoprotein particle profiles is not known. Here we examine cross-sectional associations between objectively measured physical activity and sedentary time with serum markers of lipoprotein metabolism.

Methods: Our cohort included 880 children (49.0% girls, mean age 10.2 years). Physical activity intensity and time spent sedentary were measured objectively using accelerometers. 30 measures of lipoprotein metabolism were quantified using nuclear magnetic resonance spectroscopy. Multiple linear regression models adjusted for age, sex, sexual maturity and socioeconomic status were used to determine associations of physical activity and sedentary time with lipoprotein measures. Additional models were adjusted for adiposity. Isotemporal substitution models quantified theoretical associations of replacing 30 minutes of sedentary time with 30 minutes of moderate- to vigorous-intensity physical activity (MVPA).

Results: Time spent in MVPA was associated with a favourable lipoprotein profile independent of sedentary time. There were inverse associations with a number of lipoprotein measures, including most apolipoprotein B-containing lipoprotein subclasses and triglyceride measures, the ratio of total to high-density lipoprotein (HDL) cholesterol, and non-HDL cholesterol concentration. There were positive associations with larger HDL subclasses, HDL cholesterol concentration and particle size. Reallocating 30 minutes of sedentary time to MVPA had broadly similar associations. Sedentary time was only partly and weakly associated with an unfavourable lipoprotein profile.

Conclusion: Physical activity of at least moderate-intensity is associated with a favourable lipoprotein profile in schoolchildren, independent of time spent sedentary, adiposity and other confounders.

1. Introduction

Insufficient levels of physical activity are associated with a number of adverse health indicators in children and youth, including cardiometabolic risk factors and obesity.[1–4] In contrast, it is recognised that higher levels of physical activity are favourably associated with certain traditional clinical measures of lipid metabolism.[5] The mechanisms by which physical activity exerts its metabolic benefits remain poorly understood.

Advances in quantitative high-throughput serum metabolomics have enabled more comprehensive molecular profiling of lipoprotein metabolism.[6,7] Recent studies using nuclear magnetic resonance (NMR) spectroscopy have identified disparities in the associations of constituent lipoprotein subclasses with coronary heart disease (CHD) risk, long-term participation in physical activity, and obesity in adults.[8–11] Though lipoprotein subclasses have also been profiled in children,[12–14] to our knowledge no studies have explored their independent associations with sedentary time or physical activity. We examined the cross-sectional associations between objectively measured physical activity and sedentary time and 30 lipoprotein measures in a population of Norwegian schoolchildren. Reallocating time spent sedentary to physical activity has shown beneficial associations with traditional CVD risk biomarkers.[15,16] We therefore also investigated the theoretical effect of reallocating sedentary time to moderate- to vigorous-intensity physical activity (MVPA) on these novel markers.

2. Materials and methods

2.1. Study population and design

The ASK study was a seven-month cluster randomized controlled trial (RCT) to investigate the effect of a school-based physical activity intervention on academic performance and health indices in schoolchildren (https://clinicaltrials.gov. Unique identifier: NCT02132494). The methods and design of the study have been comprehensively described previously [17]. All children were in the fifth-grade of the Norwegian school system and from the Sogn and Fjordane county in western Norway. Sixty-one schools (1282 children) agreed to participate in the study. Baseline accelerometer data collection took place between April and October 2014, prior to the physical activity intervention. There were no differences in pupils' physical activity or sedentary time between the intervention or control schools at either baseline or follow-up.[18] Hence, in the present study all participating pupils' baseline data is pooled as a cohort.

2.2. Ethics

The Regional Committee for Medical Research Ethics approved the study protocol. Procedures and methods abide by the World Medical Association's Declaration of Helsinki.[19] Written consent was obtained from each child's parent or legal guardian and from school authorities prior to testing.

2.3. Physical activity and sedentary time

Physical activity and sedentary time were measured using the ActiGraph GT3X+ triaxial accelerometer (ActiGraph LLC, Pensacola, Florida, USA). The children wore the accelerometer on their right hip, except during water-based activities or sleep for seven consecutive days. Monitor wear time of ≥480 minutes accumulated between 0600 and 0000 is considered a valid day. Non-wear time is defined as ≥20 minutes of zero counts.[20]

Accelerometer data was collected and analysed using 10-second epochs. The accelerometer data were processed using KineSoft analytical software version 3.3.80 (KineSoft, Loughborough, United Kingdom). The physical activity outcomes are min·d^{-1} in sedentary time (≤100 counts·min^{-1}), light-intensity physical activity (LPA; >100 to <2296 counts·min^{-1}), and MVPA (≥2296 counts·min^{-1}), classified using the Evenson cut points.[21,22]

2.4. Anthropometry and maturity

Body mass (weight, 0.1kg) was measured using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany). A portable stadiometer (Seca 217, SECA GmbH, Hamburg, Germany) was used to assess stature (height, 0.1cm); the child facing forward, shoes removed. Two measurements of each child's waist circumference were taken using an ergonomic circumference measuring tape (Seca 201, SECA GmbH, Hamburg, Germany). If the difference of the two measurements exceeded 1cm, a third measurement was taken. The mean of the two measurements with the least difference was used for analysis. Waist circumference has been shown to be highly correlated with both total fat mass and trunk fat measured using dual x-ray absorptiometry (DXA).[23]

The children self-assessed their genital and pubic hair development (girls also assessed their breast development) according to Tanner stages and using a standardised scale of colour images accompanied by brief text descriptions of each stage.[24] The assessments took place in a private room, accompanied by a researcher of the same sex to ensure the comfort of each child. Low frequencies were recorded in stages 4 and 5 for girls and boys, and were therefore combined with the Tanner stage 3 category. For statistical analysis, girls were assigned a single score, which corresponded to the higher of their reported Tanner stage for pubic hair and breast development.

2.5. Socioeconomic status

Socioeconomic status (SES) was quantified as the highest level of educational attainment of either a child's mother or father, whichever was higher. This information was collected using a self-report questionnaire designed for the ASK study and completed by each parent. There were six categories of educational level completed. Low frequencies were recorded in the four lowest SES categories and were combined accordingly. Hence, three categories are used in the present analysis: i) upper secondary school, ii) less than four years of college/university, iii) equal to or more than four years of college/university.

2.6. Blood sample collection and metabolite measurement

Overnight fasting blood samples for each child were drawn from the antecubital vein, between 0800 and 1000 by a trained nurse or phlebotomist. Serum samples were stored in cryotubes at -80°C until analysis. Baseline sample collection took place between August and September 2014, prior to the physical activity intervention.

2.7. Quantification of lipoprotein measures

NMR spectra were recorded on a Bruker Avance III 600MHz spectrometer, equipped with a QCI CryoProbe and automated sample changer (SampleJet) (Bruker BioSpin GmbH, Karlsruhe, Germany). The standard operating procedure as described by Dona et al. [25] was applied.

Frozen serum samples were thawed at room temperature for approximately one hour. Aliquots of 120µL were carefully mixed with equal amounts of phosphate buffer in Eppendorf tubes, and transferred to 3mm SampleJet tubes by syringe.[25] A fill height of 4cm was used amounting to approximately 180µL.

One-dimensional 1H NMR spectra were recorded at 310K, using the *noesygppr1d* pulse sequence for water suppression. Relaxation delay and mixing time were set to 4 seconds and 10ms respectively, with a low-power (25Hz) continuous-wave pulse centred at the water frequency during both delays. A total of 32 scans were recorded, using 96k data points and a spectral width of 30ppm (18 028.846Hz). A fixed receiver gain of 90.5 was used. Line broadening of 0.3Hz was applied prior to Fourier transformation. The spectra were processed to a total of 131 072 data points and automatically phased using Bruker program *apk00.noe*. For quantification, an ERETIC signal was added to the spectrum at 15ppm, using the PULCON principle.[26,27] The spectra were imported to MATLAB (MathWorks, Natick, MA, USA), scaled to the ERETIC signal and aligned to the lactate doublet at 1.32ppm.

After aligning the spectra to the lactate shifts and normalizing to the QREF-signal, we selected the shift regions describing the peaks at 1.3ppm (approx. 900 shifts) and 0.9ppm (approx. 440 shifts). These regions can provide quantitative information about the lipoprotein subclasses.[28] Without any further pretreatment, these spectral regions were selected as explanatory variables to partial least squares (PLS) modelling with subclass concentrations of cholesterol and triglycerides determined by the high-performance liquid chromatography (HPLC) as response variables.[29,30] Furthermore, we calculated PLS models for particle concentrations (particle numbers) of each lipoprotein subclass.[31] A total of 106 serum samples were randomly selected to be analysed by both NMR and HPLC and used for PLS modelling. For triglyceride concentrations of subclasses only the spectral shift region describing the peak at 1.3ppm was used. For particle and cholesterol concentrations of subclasses both windows were used. Individual PLS models with optimal prediction ability were calculated for all subclasses using a Monte-Carlo resampling approach.[32] From these models, we predicted the concentrations of 20 lipoprotein subclasses determined for both triglycerides and cholesterol individually and combined for the whole cohort. Similarly, particle concentrations were predicted for the 20 subclasses for the whole cohort.

Following the procedure of Lin et al.,[33] we calculated total particle concentrations of 20 lipoprotein subclasses, then reduced these to 15. We kept the three large very low-density lipoprotein (VLDL) subclasses distinct given the excellent resolution and accuracy achieved through the NMR spectroscopy analysis. We obtained total cholesterol and triglyceride concentrations for chylomicrons (CM), VLDL, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) subclasses. We calculated non-HDL cholesterol by subtracting the HDL cholesterol concentration from total cholesterol concentration, total to HDL cholesterol ratio, and obtained average particle diameters for VLDL, LDL, and HDL particles. In addition to quantification using NMR spectroscopy, we used standard clinical chemistry methods to

measure serum concentrations of total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol was calculated using the Friedewald formula.[34]

2.8. Statistical analysis

Descriptive data are presented as mean and standard deviation (SD), median and interquartile range [IQR] for skewed data, frequency (*n*) and proportion (%). We performed between-sex comparisons of continuous variables using independent samples Student's *t*-tests. For the categorical variables parents' education and sexual maturity, we used a 2-degree of freedom (df) Chi-square (X^2) test for between-sex comparisons. We calculated correlation coefficients for four biochemical measures (total, LDL and HDL cholesterol, and total triglycerides) measured by both clinical chemistry and NMR spectroscopy.

We visually assessed residual distributions using graphical methods and interpreted statistics. We examined associations between sedentary time and physical activity variables (i.e. LPA and MVPA) as exposure variables and each lipoprotein measure as the outcome using multiple linear regression for normally distributed lipoprotein measures (median regression for skewed measures). We modelled these associations as follows: First, models were adjusted for monitor wear time, sex, sexual maturity and SES. Second, we further adjusted model 1 for adiposity (i.e. waist circumference). Third, we mutually adjusted MVPA for sedentary time and vice versa, also adjusting for the same covariates as in models 1 and 2. Prior to regression, we scaled the lipoprotein measures to SD units.

We report regression coefficients and 95% confidence intervals (CIs) for all lipoprotein measures. Each regression coefficient represents a SD unit change in lipoprotein measure per unit increment in physical activity variable. We defined a one-unit increment in sedentary time or physical activity as 30 minutes. We standardised to 30 minutes time spent in MVPA, LPA, and sedentary time. For example, a coefficient of 0.2 for large HDL particle concentration indicates that 30 minutes of MVPA is associated with a 0.2 SD unit increment in large HDL particle concentration.

We used isotemporal substitution models to examine the effect of replacing time spent sedentary with an equal amount of MVPA. An isotemporal substitution analysis simultaneously models both the activity being performed and the activity being replaced in an equal time-exchange manner, whilst holding other activity types constant.[35] For example, by excluding sedentary time from a regression model that keeps MVPA, LPA and monitor wear time constant, the coefficient obtained for MVPA demonstrates the theoretical effect of replacing sedentary time with a specified amount of MVPA. Hence, the regression coefficients of our model represent the SD unit change in each lipoprotein measure for a 30-minute substitution of MVPA replacing 30 minutes of sedentary time. Our primary model was adjusted for monitor wear time, sex, sexual maturity and SES. We additionally adjusted for adiposity in a separate model.

Two sensitivity analyses were performed for each model. Firstly, we restricted inclusion to those children with at least four valid days of physical activity data. Secondly, we removed influential observations from each model, identified using a cut-off Cook's distance >4/*n*.

We applied multiple testing correction using false discovery rate (FDR) estimation to each regression analysis, implemented using the Benjamini-Hochberg procedure.[36] We consider 2-

sided p values <0.05 as evidence against the null hypothesis for both the FDR-corrected p values of the regression analyses and uncorrected p values for all other statistical analyses.
We performed all analyses using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). In addition to the base R software, additional packages used included: **broom**, **car**, **moments**, **quantreg**, and a number of packages from the **tidyverse** suite.

3. Results

3.1. Baseline characteristics

Table 1 shows the characteristics of the children included in our study. Valid blood samples were available for 1056 children. Of these, complete baseline data was available for 880 children, and they comprise the cohort for the present analyses. Those with complete data accumulated more LPA ($p = 0.02$), had higher clinical chemistry measures of total cholesterol ($p = 0.02$) and HDL cholesterol ($p = 0.02$), and lower triglycerides ($p = 0.02$) than those 176 children with missing data. In our analytical sample, we found between-sex differences for the clinical chemistry measure of HDL cholesterol ($p = 0.02$), sexual maturity, total physical activity, MVPA, and clinical chemistry measure of triglycerides (all p <0.01).

3.2. Comparison of lipid measurement techniques

All four clinical chemistry measures were strongly correlated with the NMR spectroscopy-derived values (Supplementary Material Figure 1).

3.3. Associations between intensity of physical activity and lipoprotein measures

Figure 1 shows the associations between a 30-minute difference in MVPA and the 30 lipoprotein measures. In the model not adjusted for adiposity, there were inverse associations with the particle concentrations of CM and all VLDL subclasses, except small VLDL, and small LDL. The positive associations with the larger HDL subclasses were marked. There were inverse associations between MVPA and the cholesterol concentrations of CM and VLDL, the ratio of total to HDL cholesterol, and a positive association with HDL cholesterol. Time spent in MVPA was inversely associated with total, CM and VLDL triglyceride concentration. There was an inverse association with average VLDL particle size, and positive associations with average LDL and HDL particle size. Adjusting for adiposity attenuated a number of the associations. However, MVPA was associated with 12 lipoprotein measures independent of adiposity. LPA was not associated with any lipoprotein measures (Supplementary Material Figure 2).

3.4. Associations between sedentary time and lipoprotein measures

Figure 2 shows the associations between a 30-minute difference in time spent sedentary and the 30 lipoprotein measures. In the model not adjusted for adiposity, there were positive associations with the particle concentrations of VLDL L2 and L3, the cholesterol concentration of CM, and average VLDL particle size. There was an inverse association with average LDL particle size. Sedentary time was not associated with any measures independent of adiposity.

3.5. Isotemporal substitution of MVPA for sedentary time

Reallocation of 30 minutes sedentary time to an additional 30 minutes of MVPA daily produced a near identical pattern of associations with the 30 lipoprotein measures as in the single activity MVPA model (Figure 3). In addition to those that were associated in the single activity model, substitution of MVPA was inversely associated with the particle concentration of the very small LDL subclass, and non-HDL cholesterol concentration. A number of the associations were independent of adiposity.

3.6. Independent associations between moderate- to vigorous-intensity physical activity, sedentary time and lipoprotein measures

Adjustment for sedentary time for the associations between MVPA and lipoprotein measures showed a broadly similar pattern of associations as without adjustment (Figure 4).

A comparison of the adiposity-adjusted MVPA single activity model with and without adjustment for sedentary time is presented in Supplementary Material Figure 3. Adjustment for MVPA in the model examining the associations between sedentary time and the lipoprotein measures showed no independent associations (Supplementary Material Figure 4 and Figure S5).

3.7. Sensitivity analyses

The association patterns of our models remained similar for each analysis when restricting included children to those 841 individuals (410 girls) that had at least four valid days of accelerometer wear data. When repeating each analysis having excluded influential observations, identified as those observations with a Cook's distance >4/*n*, the patterns of associations remained unaltered (data not shown).

4. Discussion

In our cohort of healthy, Norwegian schoolchildren, time spent in MVPA is favourably associated with a number of lipoprotein measures independent of time spent sedentary and adiposity. Our results support previous work investigating different physical activity intensities and traditional clinical chemistry measures of lipid metabolism.[37,38] The direction of association for time spent in MVPA with many of the lipoprotein measures are consistent with changes reported by Sarzynski et al. [39] in their meta-analysis of exercise interventions in adults. The pattern of associations shown in the MVPA isotemporal substitution model suggest that these potential benefits could be achieved within a waking day by reallocation of time from sedentary behaviours. Similar theoretical effects of reallocating sedentary time to MVPA in children produced favourable changes in traditional cardiometabolic risk factors.[16] In our population, reallocation to 30 additional minutes of daily MVPA corresponds to an average relative increase in MVPA of 40%, which, though challenging in an already active population, is feasible. The associations between sedentary time, LPA and lipoprotein profile are negligible,[38,40] and likely mediated by adiposity and MVPA.

There are a limited number of studies investigating physical activity and NMR spectroscopy-derived lipoprotein measures. Kujala et al. [10] reported a favourable lipoprotein profile for adults that self-reported as persistently physically active compared to those who were inactive. Also in adults, Aadland et al. [11] reported complementary findings to ours for individuals that spent

a greater proportion of awake time in MVPA. Our observations extend those previously observed in adults to healthy children.

In a recent paper, Holmes et al. [41] investigated associations between a number of lipoprotein measures quantified using NMR spectroscopy and risk of myocardial infarction (MI) and stroke in adults. They reported significantly increased odds of an event for 1 SD greater particle and cholesterol concentrations of all apolipoprotein B-containing lipoprotein subclasses, and with triglyceride concentration in most subclasses. Many of the lipoprotein measures associated with increased odds in that study are inversely associated with time spent in MVPA in our study. Therefore, increasing levels of physical activity may reduce CVD risk, though we are cautious extrapolating the findings of our single study in children to clinical endpoints in adults.

Strengths of our study include the reasonably large sample size, which enabled us to investigate a number of individual lipoprotein measures. Objective measurement of physical activity reduces the potential for misclassification compared to self-reported assessment. Other strengths include high compliance with physical activity measurement, and adjustment for a number of confounders, including adiposity and mutual adjustment for MVPA and sedentary time. The decision to include all children with at least one valid day of accelerometer data is supported by the unchanged pattern of associations shown in the sensitivity analyses that excluded children with less than four valid days.

We acknowledge some limitations of our study. The data is cross-sectional, thus limiting our ability to attribute causality. Though there is potential bidirectional causation between adiposity and physical activity levels, it is unlikely that the lipoprotein measures themselves directly influence physical activity. For example, given that many of the associations remain after adjustment for adiposity in our MVPA model, suggests that physical activity affects these metabolic markers independent of adiposity. We acknowledge that we cannot exclude the potential for residual confounding from unmeasured variables such as dietary composition and genotype.

Though there are a number of advantages to objective measurement of physical activity using accelerometers [42] including their popularity, which facilitates comparisons between cohorts and data pooling,[43–45] well-known limitations remain. For instance, the inability of accelerometry to accurately assess the intensity of certain activities, like swimming or bicycling. Further, a week of objective measurement may not reflect habitual activity patterns. A previous study in children reported intraclass correlation coefficients of approximately 0.5 for serial objectively measured physical activity.[46] However, if we assume that the within-individual measurement error is random, it is likely that the observed associations are attenuated and possible, therefore, that the magnitudes be twice as strong as reported here. The marginal associations reported for LPA in our results could be due to known issues of misclassification with sedentary time when using accelerometer cut-points.[47] Lastly, given that our sample is from a particular geographical region within Norway, the generalisability of our findings to other populations is limited.

4.1. Conclusion

Physical activity of at least moderate-intensity shows broadly favourable associations with lipoprotein metabolism, independent of time spent sedentary. These associations are somewhat attenuated by, but mostly independent of, adiposity and suggest a combination of increased physical activity coupled with approaches to reduce adiposity are likely to be more beneficial than unidimensional interventions. Theoretically, these benefits could be achieved by reallocating 30 minutes of sedentary time to moderate- to vigorous-intensity physical activity each day. Larger, longitudinal studies of more diverse populations are required to establish the broader applicability of our findings, investigate their stability into adulthood and potential associations with clinical endpoints.

References

1 Andersen LB, Riddoch C, Kriemler S, Hills AP, Hills A. Physical activity and cardiovascular risk factors in children. *British journal of sports medicine* 2011; **45**: 871–6.

2 Skrede T, Stavnsbo M, Aadland E, *et al.* Moderate-to-vigorous physical activity, but not sedentary time, predicts changes in cardiometabolic risk factors in 10-y-old children: the Active Smarter Kids Study. *The American journal of clinical nutrition* 2017; **105**: 1391–8.

3 Poitras VJ, Gray CE, Borghese MM, *et al.* Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 2016; **41**: S197-239.

4 Andersen LB, Harro M, Sardinha LB, *et al.* Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *Lancet (London, England)* 2006; **368**: 299–304.

5 Physical Activity Guidelines Advisory Committee. Physical Activity Guidelines Advisory Committee report, 2008. Washington, DC: US Department of Health and Human Services, 2008 DOI:10.1111/j.1753-4887.2008.00136.x.

6 Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technology. *American Journal of Epidemiology* 2017; : 1–13.

7 Soininen P, Kangas AJ, Würtz P, *et al.* High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *The Analyst* 2009; **134**: 1781–5.

8 Akinkuolie AO, Paynter NP, Padmanabhan L, Mora S. High-density lipoprotein particle subclass heterogeneity and incident coronary heart disease. *Circulation Cardiovascular quality and outcomes* 2014; **7**: 55–63.

9 Magkos F, Mohammed BS, Mittendorfer B. Effect of obesity on the plasma lipoprotein subclass profile in normoglycemic and normolipidemic men and women. *International journal of obesity (2005)* 2008; **32**: 1655–64.

10 Kujala UM, Mäkinen V-P, Heinonen I, *et al.* Long-term leisure-time physical activity and serum metabolome. *Circulation* 2013; **127**: 340–8.

11 Aadland E, Andersen JR, Anderssen SA, Kvalheim OM. Physical activity versus sedentary behavior: Associations with lipoprotein particle subclass concentrations in healthy adults. *PLoS ONE* 2013; **8**: 1–9.

12 Rajalahti T, Lin C, Mjøs SA, Kvalheim OM. Serum fatty acid and lipoprotein subclass concentrations and their associations in prepubertal healthy Norwegian children. *Metabolomics : Official journal of the Metabolomic Society* 2016; **12**: 81.

13 Resaland GK, Rajalahti T, Aadland E, Kvalheim OM. Strong association between cardiorespiratory fitness and serum lipoprotein subclass pattern in prepubertal healthy children. *Scandinavian journal of medicine & science in sports* 2017; : 1–8.

14 Würtz P, Wang Q, Kangas AJ, *et al.* Metabolic signatures of adiposity in young adults: Mendelian randomization analysis and effects of weight change. *PLoS medicine* 2014; **11**: e1001765.

15 Buman MP, Winkler EAH, Kurka JM, *et al.* Reallocating time to sleep, sedentary behaviors, or active behaviors: associations with cardiovascular disease risk biomarkers, NHANES 2005-2006. *American journal of epidemiology* 2014; **179**: 323–34.
16 Hansen BH, Anderssen SA, Andersen LB, *et al.* Cross-Sectional Associations of Reallocating Time Between Sedentary and Active Behaviours on Cardiometabolic Risk Factors in Young People: An International Children's Accelerometry Database (ICAD) Analysis. *Sports medicine (Auckland, NZ)* 2018; published online April 6. DOI:10.1007/s40279-018-0909-1.
17 Resaland GK, Moe VF, Aadland E, *et al.* Active Smarter Kids (ASK): Rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on children's academic performance and risk factors for non-communicable diseases. *BMC public health* 2015; **15**: 709.
18 Resaland GK, Aadland E, Moe VF, *et al.* Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. *Preventive medicine* 2016; **91**: 322–8.
19 World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013; **310**: 2191–4.
20 Esliger DW, Copeland JL, Barnes JD, Tremblay MS. Standardizing and optimizing the use of accelerometer data for free-living physical activity monitoring. *Journal of Physical Activity and Health* 2005; **2**: 366–83.
21 Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. *Journal of sports sciences* 2008; **26**: 1557–65.
22 Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of accelerometer cut points for predicting activity intensity in youth. *Medicine and Science in Sports and Exercise* 2011; **43**: 1360–8.
23 Boeke CE, Oken E, Kleinman KP, Rifas-Shiman SL, Taveras EM, Gillman MW. Correlations among adiposity measures in school-aged children. *BMC pediatrics* 2013; **13**: 99.
24 Carel J, Léger J. Precocious Puberty. *New England Journal of Medicine* 2008; **358**: 2366–77.
25 Dona AC, Jiménez B, Schäfer H, *et al.* Precision high-throughput proton NMR spectroscopy of human urine, serum, and plasma for large-scale metabolic phenotyping. *Analytical chemistry* 2014; **86**: 9887–94.
26 Akoka S, Barantin L, Trierweiler M. Concentration Measurement by Proton NMR Using the ERETIC Method. *Analytical chemistry* 1999; **71**: 2554–7.
27 Wider G, Dreier L. Measuring protein concentrations by NMR spectroscopy. *Journal of the American Chemical Society* 2006; **128**: 2571–6.
28 Mihaleva V V, van Schalkwijk DB, de Graaf AA, *et al.* A systematic approach to obtain validated partial least square models for predicting lipoprotein subclasses from serum NMR spectra. *Analytical chemistry* 2014; **86**: 543–50.
29 Wold S, Ruhe A, Wold H, Dunn, III WJ. The Collinearity Problem in Linear Regression. The Partial Least Squares (PLS) Approach to Generalized Inverses. *SIAM Journal on Scientific and Statistical Computing* 1984; **5**: 735–43.

30 Okazaki M, Usui S, Ishigami M, *et al.* Identification of unique lipoprotein subclasses for visceral obesity by component analysis of cholesterol profile in high-performance liquid chromatography. *Arteriosclerosis, thrombosis, and vascular biology* 2005; **25**: 578–84.
31 Okazaki M, Yamashita S. Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using 'Spherical Particle Model'. *Journal of oleo science* 2016; **65**: 265–82.
32 Kvalheim OM, Arneberg R, Grung B, Rajalathi T. Determination of optimum number of components in partial least squares regression from distributions of the root-mean-squared error obtained by Monte Carlo resampling. *Journal of Chemometrics* 2018; **32**: e2293.
33 Lin C, Rajalahti T, Mjøs SA, Kvalheim OM. Predictive associations between serum fatty acids and lipoproteins in healthy non-obese Norwegians: implications for cardiovascular health. *Metabolomics : Official journal of the Metabolomic Society* 2016; **12**: 6.
34 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry* 1972; **18**: 499–502.
35 Mekary RA, Willett WC, Hu FB, Ding EL. Isotemporal substitution paradigm for physical activity epidemiology and weight change. *American journal of epidemiology* 2009; **170**: 519–27.
36 Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)* 1995; **57**: 289–300.
37 LeBlanc AG, Janssen I. Dose-response relationship between physical activity and dyslipidemia in youth. *The Canadian journal of cardiology* 2010; **26**: 201–5.
38 Ekelund U, Luan J, Sherar LB, *et al.* Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. *JAMA* 2012; **307**: 704–12.
39 Sarzynski MA, Burton J, Rankinen T, *et al.* The effects of exercise on the lipoprotein subclass profile: A meta-analysis of 10 interventions. *Atherosclerosis* 2015; **243**: 364–72.
40 Barnett TA, Kelly AS, Young DR, *et al.* Sedentary Behaviors in Today's Youth: Approaches to the Prevention and Management of Childhood Obesity: A Scientific Statement From the American Heart Association. *Circulation* 2018; **138**: e142–e159.
41 Holmes M V., Millwood IY, Kartsonaki C, *et al.* Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *Journal of the American College of Cardiology* 2018; **71**: 620–32.
42 Strath SJ, Kaminsky LA, Ainsworth BE, *et al.* Guide to the assessment of physical activity: Clinical and research applications: A scientific statement from the American Heart association. *Circulation* 2013; **128**: 2259–79.
43 Cooper AR, Goodman A, Page AS, *et al.* Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). *The international journal of behavioral nutrition and physical activity* 2015; **12**: 113.
44 Oliver M, Schofield GM, Kolt GS. Physical activity in preschoolers: understanding prevalence and measurement issues. *Sports medicine (Auckland, NZ)* 2007; **37**: 1045–70.
45 Corder K, Ekelund U, Steele RM, Wareham NJ, Brage S. Assessment of physical activity in youth. *Journal of applied physiology (Bethesda, Md : 1985)* 2008; **105**: 977–87.

46 Aadland E, Andersen LB, Skrede T, Ekelund U, Anderssen SA, Resaland GK. Reproducibility of objectively measured physical activity and sedentary time over two seasons in children; Comparing a day-by-day and a week-by-week approach. *PloS one* 2017; **12**: e0189304.
47 Hänggi JM, Phillips LRS, Rowlands A V. Validation of the GT3X ActiGraph in children and comparison with the GT1M ActiGraph. *Journal of science and medicine in sport* 2013; **16**: 40–4.

Conflict of interest
The Authors declare that there is no conflict of interest.

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Author contributions
PRJ and UE conceived and designed the analysis. GKR and EA collected the data. TR, OMK, TFB, TA and EA contributed data or analysis tools. PRJ performed the analysis. PRJ drafted all versions of the manuscript and all other authors critically revised and approved the final version.

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Figure legends

Figure 1. Cross-sectional associations of time spent in MVPA with 30 serum lipoprotein measures (n = 880)

The associations were adjusted for monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in MVPA. Filled circles are FDR-corrected p value <0.05. Error bars are 95% CIs. CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 2. Cross-sectional associations of sedentary time with 30 serum lipoprotein measures (n = 880)

The associations were adjusted for monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in sedentary time. Filled circles are FDR-corrected p value <0.05. Error bars are 95% CIs. CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 3. Cross-sectional associations of 30 serum lipoprotein measures with an isotemporal substitution of 30 minutes time spent in MVPA for 30 minutes of sedentary time (n = 880)

The associations were adjusted for monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure for a 30-minute reallocation of activity. Filled circles are FDR-corrected p value <0.05. Error bars are 95% CIs. CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 4. Cross-sectional associations of time spent in MVPA with 30 serum lipoprotein measures adjusted for sedentary time (n = 880)

The associations were adjusted for sedentary time, monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in MVPA. Filled circles are FDR-corrected p value <0.05. Error bars are 95% CIs. CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density

lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 1. Baseline characteristics of the included participants

Characteristic	All	Girls	Boys	p^c
n (%)	880	431 (49.0)	449 (51.0)	
Age, years[a]	10.2 (0.3)	10.2 (0.3)	10.2 (0.3)	0.82
Parents' education, *n* (%)				
Upper secondary school	287 (32.6)	142 (32.9)	145 (32.3)	0.73
<4 years college/university	268 (30.5)	126 (29.2)	142 (31.6)	
≥4 years college/university	325 (36.9)	163 (37.8)	162 (36.1)	
Anthropometry				
Height, cm[a]	142.9 (6.8)	142.6 (6.8)	143.1 (6.7)	0.32
Weight, kg[a]	37.1 (8.1)	37.2 (8.4)	37.0 (7.8)	0.85
BMI, $kg \cdot m^{-2}$[a]	18.1 (3.0)	18.1 (3.1)	18.0 (2.9)	0.47
WC, cm[a]	62.0 (7.5)	61.5 (7.8)	62.5 (7.2)	0.07
Tanner stage, *n* (%)				
Stage 1	245 (27.8)	88 (20.4)	157 (36.4)	<0.01
Stage 2	529 (60.1)	284 (65.9)	245 (56.8)	
Stage ≥3	106 (12.0)	59 (13.7)	47 (10.9)	
Physical activity				
Wear time, $min \cdot d^{-1}$[a]	778 (58)	776 (56)	781 (60)	0.20
Total PA, $counts \cdot min^{-1}$[a]	741 (288)	696 (243)	784 (320)	<0.01
SED, $min \cdot d^{-1}$[a]	466 (58)	468 (57)	464 (58)	0.31
LPA, $min \cdot d^{-1}$[a]	233 (39)	236 (37)	231 (40)	0.08
MVPA, $min \cdot d^{-1}$[a]	76 (27)	69 (21)	83 (29)	<0.01
Clinical chemistry				
TC, $mmol \cdot L^{-1}$[a]	4.5 (0.7)	4.5 (0.7)	4.5 (0.7)	0.63
LDL C, $mmol \cdot L^{-1}$[a]	2.5 (0.6)	2.5 (0.6)	2.5 (0.7)	0.54
HDL C, $mmol \cdot L^{-1}$[a]	1.6 (0.3)	1.6 (0.3)	1.6 (0.3)	0.02[d]
TG, $mmol \cdot L^{-1}$[b]	0.7 [0.5, 0.9]	0.7 [0.6, 1.0]	0.6 [0.5, 0.8]	<0.01[e]

[a]Mean (SD).

[b]Median [IQR].

[c]*p* value between sexes derived from independent samples Student's *t*-test (continuous, normally distributed variables); 2-df Chi-square test (categorical variables).

[d]Values to 2 decimal places: 1.58 for girls, 1.63 for boys.

[e]*p* value for *t*-test shown for log(TG). Wilcoxon rank-sum test $p < 0.01$.

BMI = body mass index; HDL C = high-density lipoprotein; LDL C = low-density lipoprotein cholesterol; LPA = light-intensity physical activity; MVPA = moderate- to vigorous-intensity physical activity; PA = physical activity; SED = sedentary time; TC = total cholesterol; cholesterol; TG = triglycerides; WC = waist circumference.

Figure 1.

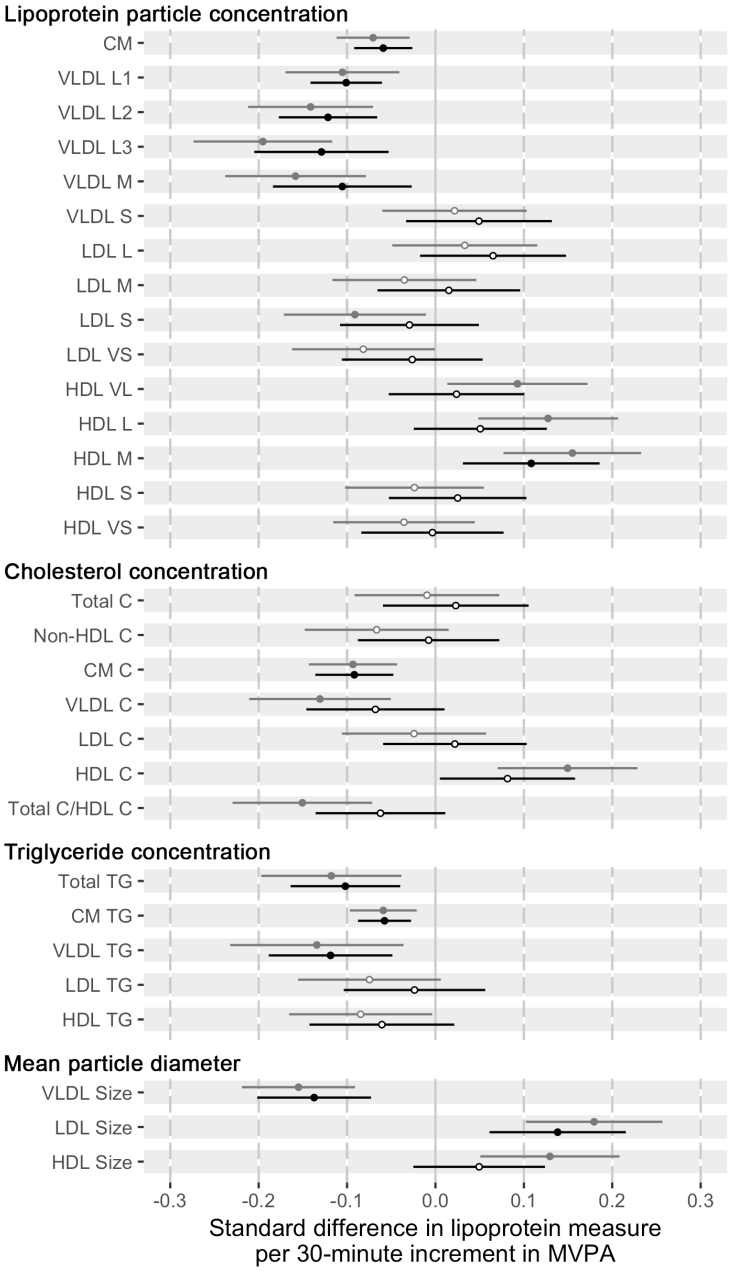

Lipoprotein particle concentration
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
CM C
VLDL C
LDL C
HDL C
Total C/HDL C
Triglyceride concentration
Total TG
CM TG
VLDL TG
LDL TG
HDL TG
Mean particle diameter
VLDL Size
LDL Size
HDL Size
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Standard difference in lipoprotein measure
per 30-minute increment in MVPA

Figure 2.

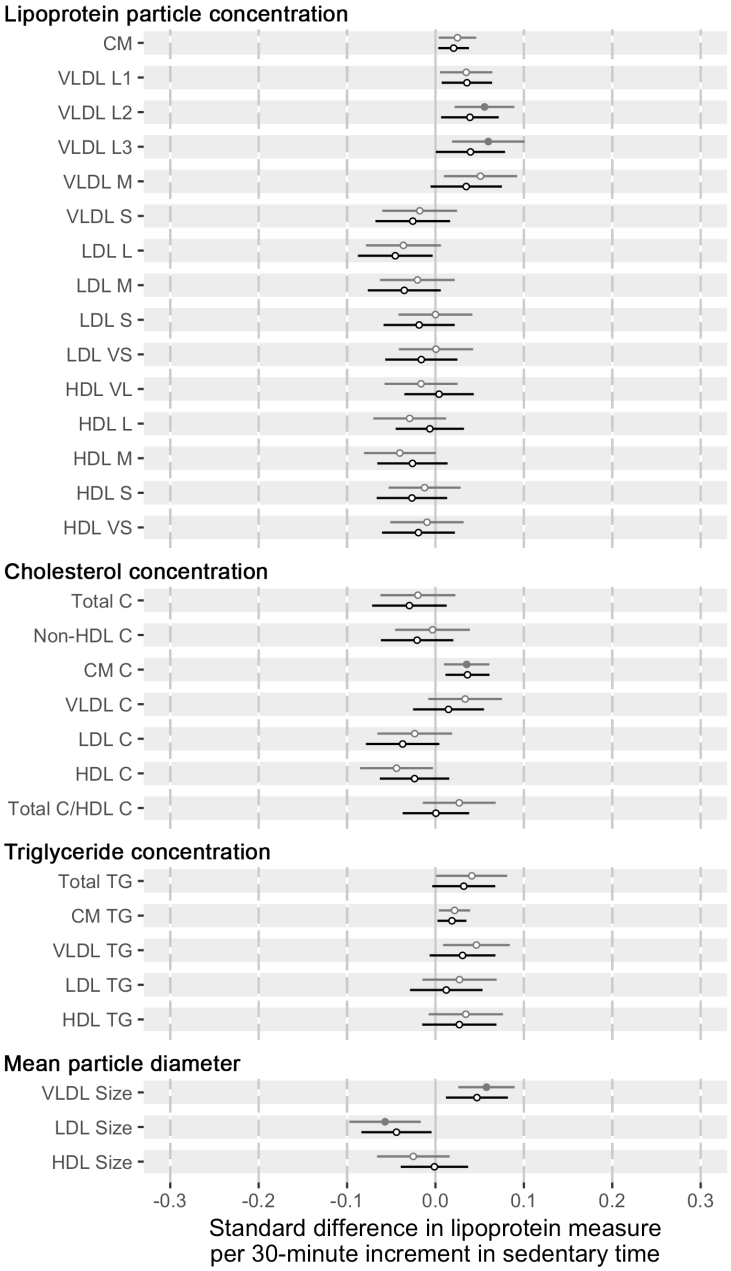

Lipoprotein particle concentration
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
CM C
VLDL C
LDL C
HDL C
Total C/HDL C
Triglyceride concentration
Total TG
CM TG
VLDL TG
LDL TG
HDL TG
Mean particle diameter
VLDL Size
LDL Size
HDL Size
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Standard difference in lipoprotein measure
per 30-minute increment in sedentary time

Figure 3.

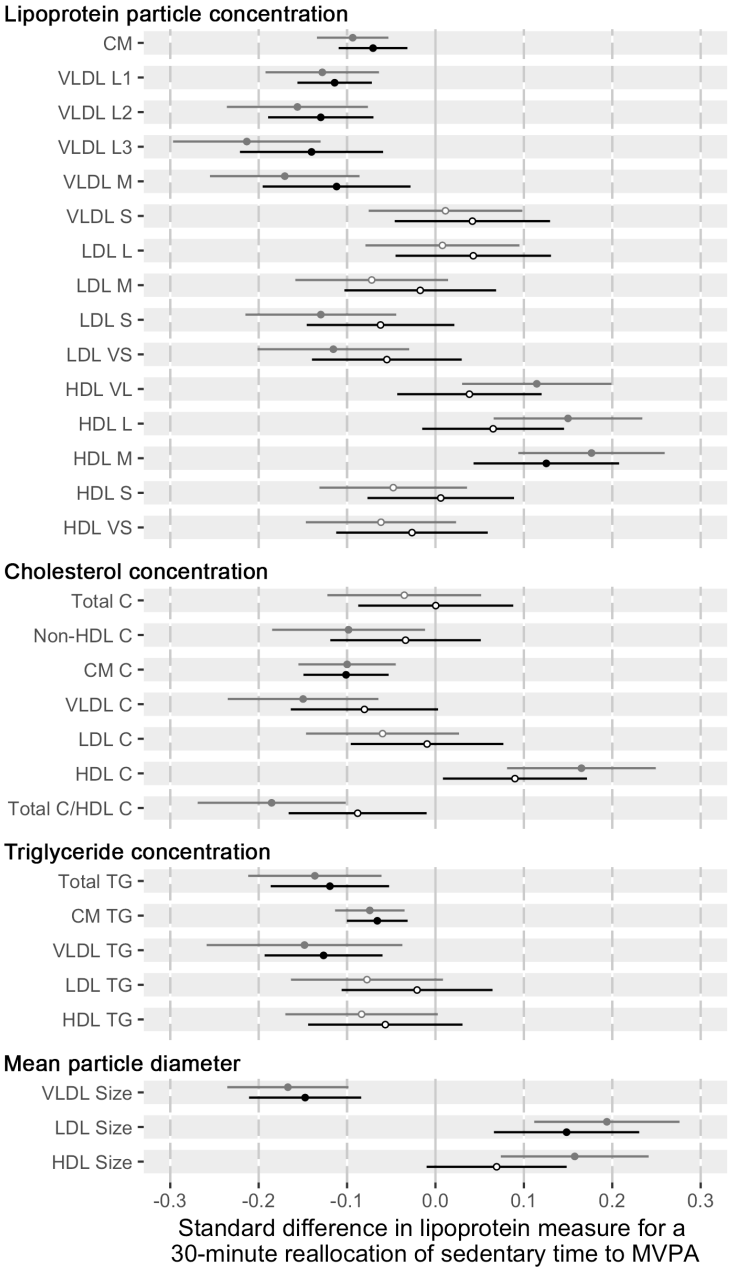
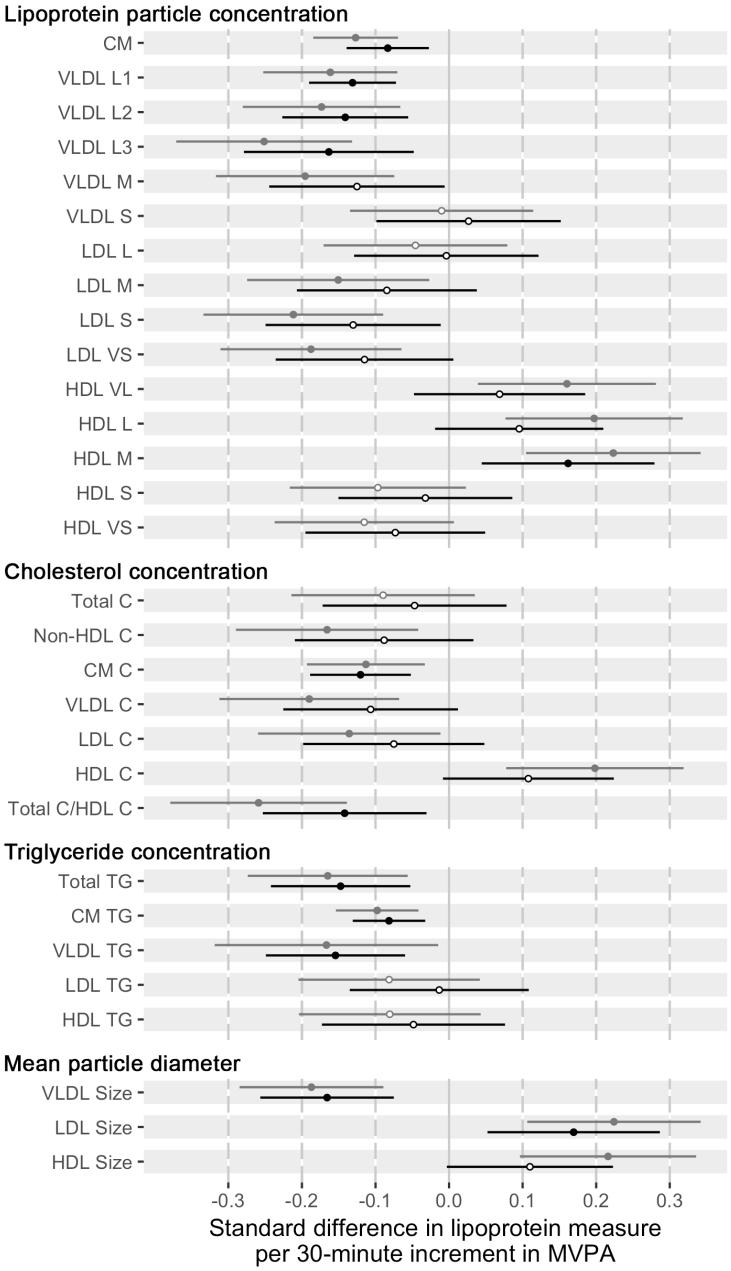
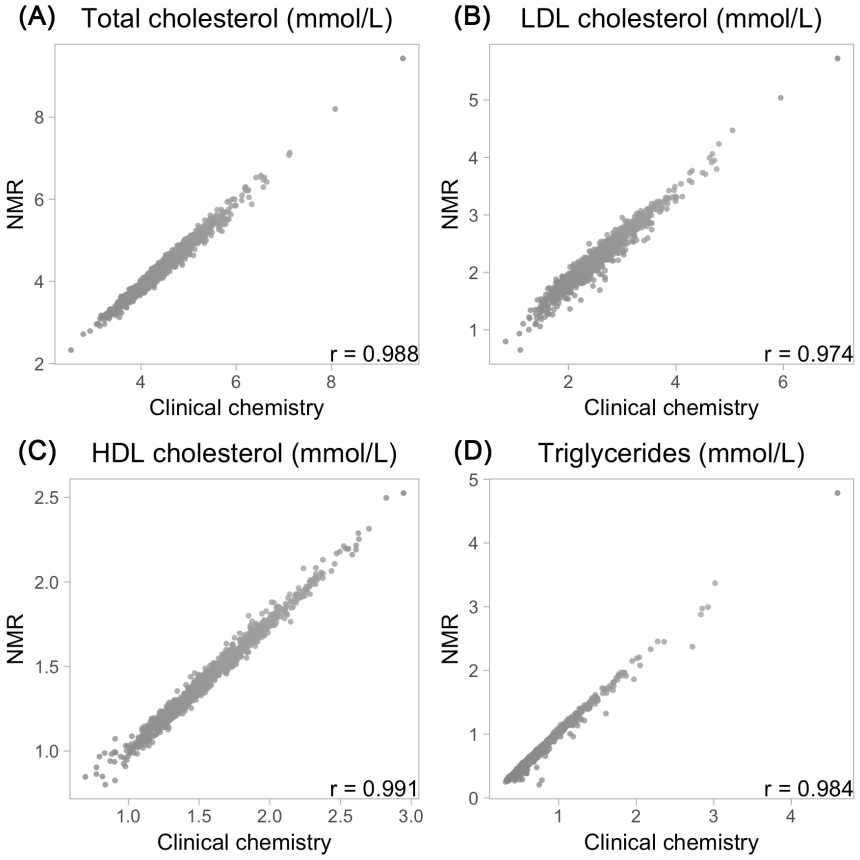

Lipoprotein particle concentration
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
CM C
VLDL C
LDL C
HDL C
Total C/HDL C
Triglyceride concentration
Total TG
CM TG
VLDL TG
LDL TG
HDL TG
Mean particle diameter
VLDL Size
LDL Size
HDL Size
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Standard difference in lipoprotein measure for a
30-minute reallocation of sedentary time to MVPA

Figure 4.


Lipoprotein particle concentration
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
CM C
VLDL C
LDL C
HDL C
Total C/HDL C
Triglyceride concentration
Total TG
CM TG
VLDL TG
LDL TG
HDL TG
Mean particle diameter
VLDL Size
LDL Size
HDL Size
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Standard difference in lipoprotein measure
per 30-minute increment in MVPA

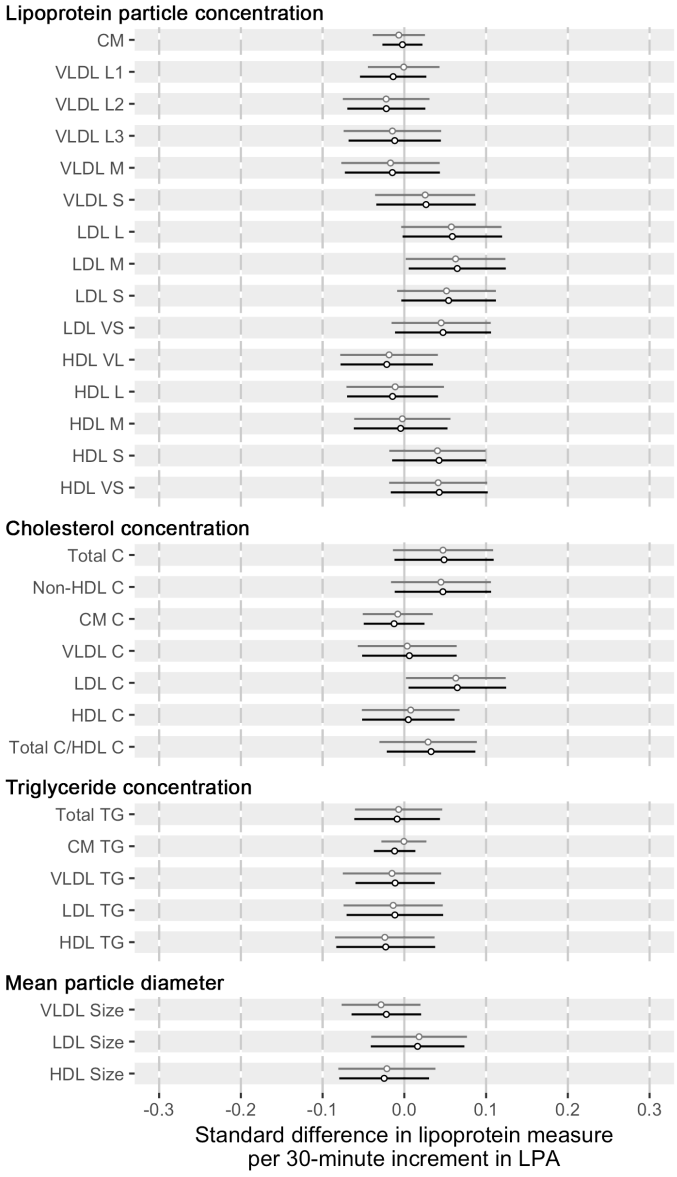
Paper I supplementary material

Figure 1. Correlation scatterplots of clinical chemistry and NMR spectroscopy measures



Values are Pearson's correlation coefficient for total (A), LDL (B), and HDL cholesterol (C); Spearman's rank correlation coefficient for triglycerides (D). Pearson's correlation coefficient for log(triglycerides) was 0.992. Abbreviations: HDL = high-density lipoprotein; LDL = low-density lipoprotein; NMR = nuclear magnetic resonance.

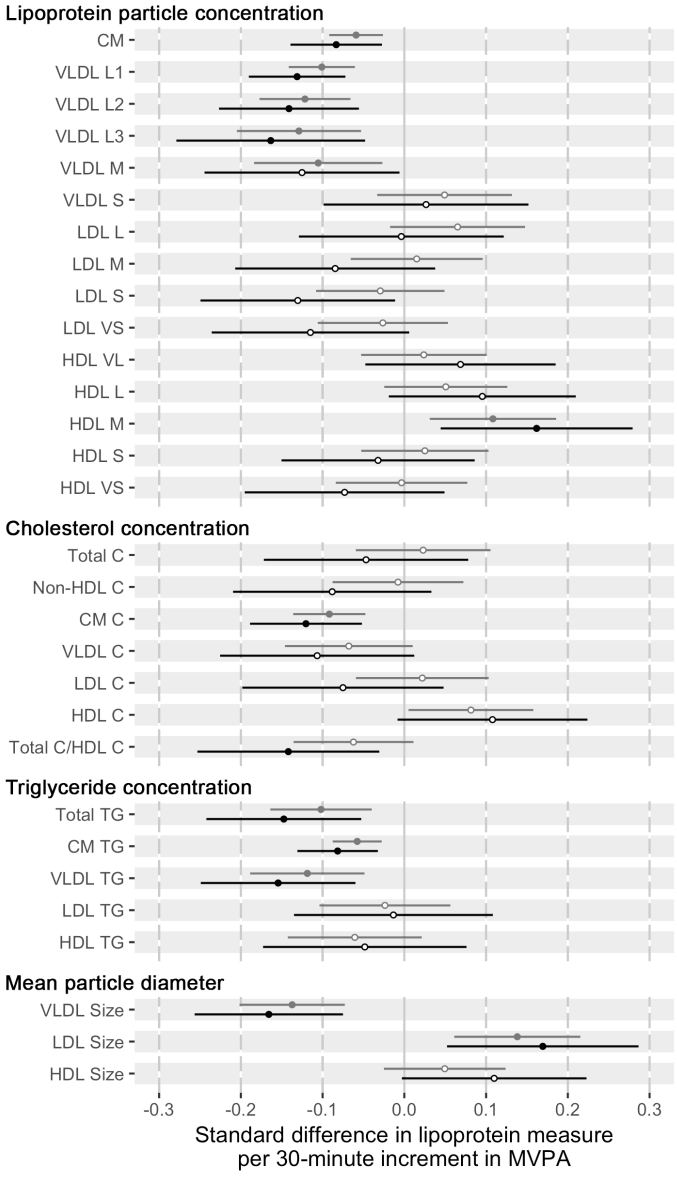
Figure 2. Cross-sectional associations of time spent in LPA with 30 serum lipoprotein measures (n = 880)



The associations were adjusted for monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in LPA. Unfilled circles are FDR-corrected p value ≥0.05. Error bars are 95% CIs.

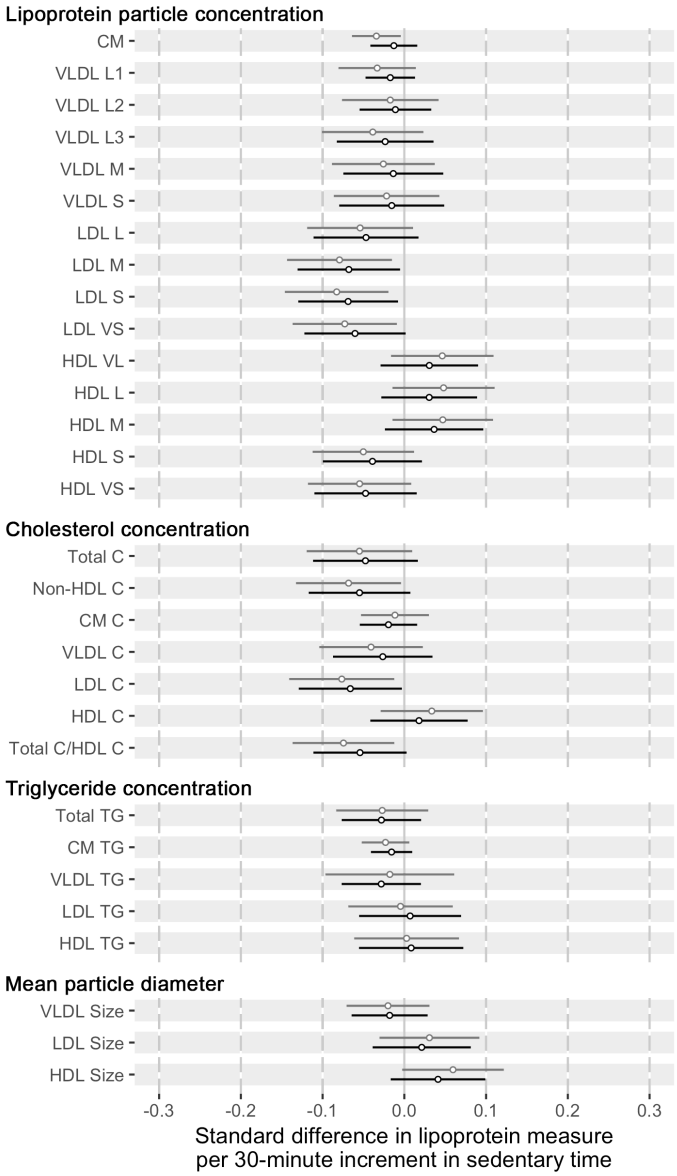
Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 3. Comparison of MVPA model adjusted for sedentary time, and unadjusted model (n = 880).



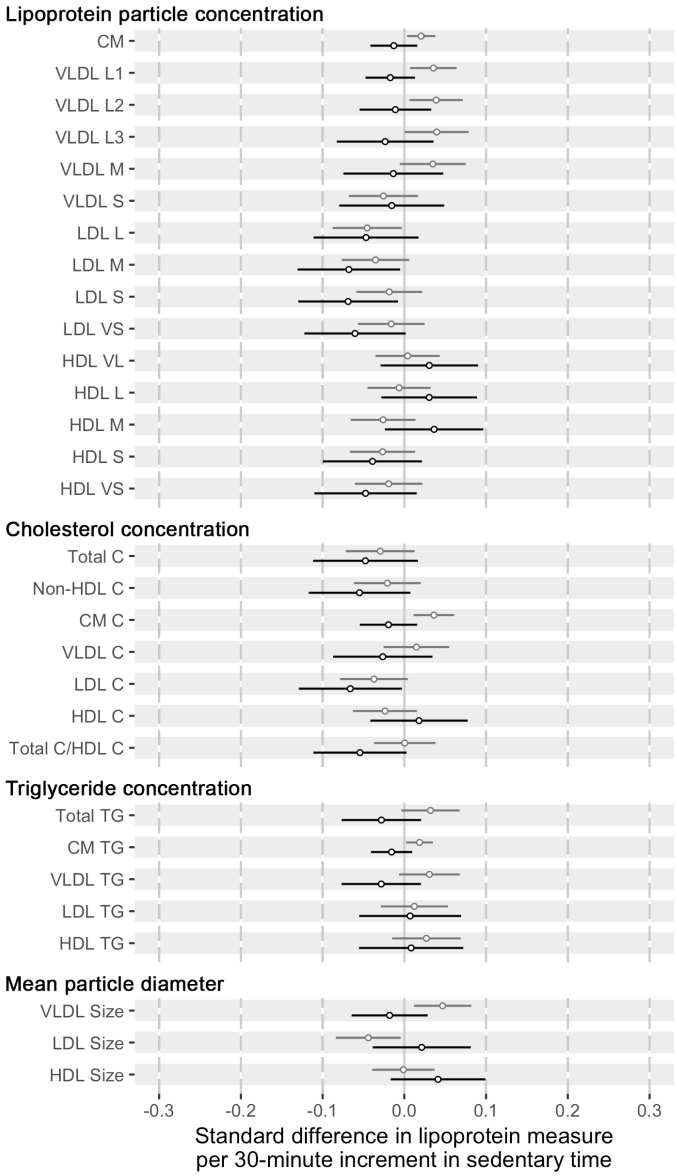
The associations were adjusted for adiposity, monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for sedentary time (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in MVPA. Filled circles are FDR-corrected p value <0.05. Error bars are 95% CIs.
Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 4. Cross-sectional associations of sedentary time with 30 serum lipoprotein measures adjusted for MVPA (n = 880).



The associations were adjusted for MVPA, monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in sedentary time. Unfilled circles are FDR-corrected p value ≥0.05. Error bars are 95% CIs.
Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Figure 5. Comparison of sedentary time model adjusted for MVPA, and unadjusted model (n = 880)



The associations were adjusted for adiposity, monitor wear time, sex, sexual maturity and SES (grey). Analyses were additionally adjusted for MVPA (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-minute increment in sedentary time. Unfilled circles are FDR-corrected p value ≥0.05. Error bars are 95% Cis.
Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 1. Mean (SD) for each lipoprotein measure (n = 1056)

Lipoprotein measure (units)	Mean (SD)	Coefficient of variation (%)
Concentration of CM particles (nmol·L^{-1})	0.243 (0.373)	153.7
Concentration of VLDL L1 particles (nmol·L^{-1})	1.04 (1.21)	115.5
Concentration of VLDL L2 particles (nmol·L^{-1})	4.41 (4.44)	100.7
Concentration of VLDL L3 particles (nmol·L^{-1})	19.4 (10.5)	54.0
Concentration of VLDL M particles (nmol·L^{-1})	29.6 (13.3)	45.1
Concentration of VLDL S particles (nmol·L^{-1})	45.1 (11.2)	24.9
Concentration of LDL L particles (nmol·L^{-1})	213 (45.3)	21.3
Concentration of LDL M particles (nmol·L^{-1})	468 (102)	21.9
Concentration of LDL S particles (nmol·L^{-1})	222 (50.6)	22.8
Concentration of LDL VS particles (nmol·L^{-1})	125 (24.7)	19.7
Concentration of HDL VL particles (nmol·L^{-1})	221 (91.3)	41.3
Concentration of HDL L particles (nmol·L^{-1})	1622 (734)	45.3
Concentration of HDL M particles (nmol·L^{-1})	4294 (599)	14.0
Concentration of HDL S particles (nmol·L^{-1})	5214 (545)	10.5
Concentration of HDL VS particles (nmol·L^{-1})	3909 (331)	8.5
Total cholesterol concentration (mmol·L^{-1})	4.35 (0.681)	15.7
Cholesterol in CM particles (mmol·L^{-1})	0.0108 (0.0138)	127.8
Cholesterol in VLDL particles (mmol·L^{-1})	0.648 (0.255)	39.3
Cholesterol in LDL particles (mmol·L^{-1})	2.22 (0.502)	22.6
Cholesterol in HDL particles (mmol·L^{-1})	1.45 (0.256)	17.6
Total triglycerides concentration (mmol·L^{-1})	0.755 (0.398)	52.7
Triglycerides in CM particles (mmol·L^{-1})	0.0258 (0.042)	163.0
Triglycerides in VLDL particles (mmol·L^{-1})	0.439 (0.31)	70.6
Triglycerides in LDL particles (mmol·L^{-1})	0.191 (0.0342)	17.9
Triglycerides in HDL particles (mmol·L^{-1})	0.104 (0.037)	35.6
Mean diameter for VLDL particles (nm)	42.6 (3.02)	7.1
Mean diameter for LDL particles (nm)	25.8 (0.137)	0.5
Mean diameter for HDL particles (nm)	10.9 (0.213)	2.0

Abbreviations: SD = standard deviation; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 2. Associations of 30 minutes' MVPA and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.0263 (–0.0417, –0.0109)	–0.022 (–0.0343, –0.00976)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.127 (–0.204, –0.0492)	–0.122 (–0.17, –0.0729)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.628 (–0.942, –0.314)	–0.54 (–0.788, –0.293)
Concentration of VLDL L3 particles (nmol·L^{-1})	–2.05 (–2.87, –1.22)	–1.35 (–2.15, –0.556)
Concentration of VLDL M particles (nmol·L^{-1})	–2.11 (–3.17, –1.05)	–1.4 (–2.45, –0.358)
Concentration of VLDL S particles (nmol·L^{-1})	0.242 (–0.675, 1.16)	0.553 (–0.373, 1.48)
Concentration of LDL L particles (nmol·L^{-1})	1.5 (–2.21, 5.21)	2.95 (–0.79, 6.69)
Concentration of LDL M particles (nmol·L^{-1})	–3.6 (–11.9, 4.72)	1.54 (–6.7, 9.79)
Concentration of LDL S particles (nmol·L^{-1})	–4.61 (–8.69, –0.541)	–1.49 (–5.47, 2.49)
Concentration of LDL VS particles (nmol·L^{-1})	–2.01 (–4, –0.0176)	–0.649 (–2.61, 1.32)
Concentration of HDL VL particles (nmol·L^{-1})	8.47 (1.22, 15.7)	2.17 (–4.83, 9.17)
Concentration of HDL L particles (nmol·L^{-1})	93.5 (35.4, 152)	37.2 (–18.1, 92.5)
Concentration of HDL M particles (nmol·L^{-1})	92.8 (46.1, 140)	65 (18.6, 111)
Concentration of HDL S particles (nmol·L^{-1})	–13 (–55.8, 29.9)	13.7 (–28.8, 56.2)
Concentration of HDL VS particles (nmol·L^{-1})	–11.8 (–38.2, 14.7)	–1.13 (–27.7, 25.5)
Total cholesterol concentration (mmol·L^{-1})	–0.00658 (–0.0623, 0.0492)	0.0157 (–0.0405, 0.0718)
Cholesterol in CM particles (mmol·L^{-1})	–0.00128 (–0.00197, –0.000598)	–0.00126 (–0.00187, –0.000656)
Cholesterol in VLDL particles (mmol·L^{-1})	–0.0333 (–0.0537, –0.0129)	–0.0173 (–0.0372, 0.00262)
Cholesterol in LDL particles (mmol·L^{-1})	–0.0122 (–0.0531, 0.0288)	0.011 (–0.0297, 0.0518)
Cholesterol in HDL particles (mmol·L^{-1})	0.0383 (0.018, 0.0586)	0.0209 (0.00129, 0.0405)
Total triglycerides concentration (mmol·L^{-1})	–0.0469 (–0.0785, –0.0153)	–0.0406 (–0.0653, –0.0159)
Triglycerides in CM particles (mmol·L^{-1})	–0.00248 (–0.00406, –0.000894)	–0.00242 (–0.00368, –0.00116)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.0416 (–0.072, –0.0112)	–0.0368 (–0.0585, –0.0151)
Triglycerides in LDL particles (mmol·L^{-1})	–0.00255 (–0.00531, 0.000211)	–0.000811 (–0.00355, 0.00192)
Triglycerides in HDL particles (mmol·L^{-1})	–0.00313 (–0.00613, –0.000135)	–0.00224 (–0.00528, 0.000788)
Mean diameter for VLDL particles (nm)	–0.468 (–0.661, –0.275)	–0.415 (–0.609, –0.22)

Mean diameter for LDL particles (nm)	0.0246 (0.014, 0.0352)	0.019 (0.00838, 0.0295)
Mean diameter for HDL particles (nm)	0.0276 (0.0108, 0.0444)	0.0105 (–0.00533, 0.0264)

Each regression analysis was adjusted for monitor wear time, SES, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SES = socioeconomic status; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 3. Associations of 30 minutes' sedentary time and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	0.00927 (0.00133, 0.0172)	0.00767 (0.0012, 0.0141)
Concentration of VLDL L1 particles (nmol·L^{-1})	0.042 (0.00633, 0.0776)	0.0429 (0.00847, 0.0773)
Concentration of VLDL L2 particles (nmol·L^{-1})	0.247 (0.0965, 0.397)	0.173 (0.0284, 0.318)
Concentration of VLDL L3 particles (nmol·L^{-1})	0.627 (0.196, 1.06)	0.416 (0.00505, 0.826)
Concentration of VLDL M particles (nmol·L^{-1})	0.679 (0.126, 1.23)	0.464 (–0.0738, 1)
Concentration of VLDL S particles (nmol·L^{-1})	–0.2 (–0.675, 0.276)	–0.288 (–0.763, 0.186)
Concentration of LDL L particles (nmol·L^{-1})	–1.64 (–3.56, 0.282)	–2.06 (–3.97, –0.143)
Concentration of LDL M particles (nmol·L^{-1})	–2.08 (–6.39, 2.23)	–3.6 (–7.83, 0.617)
Concentration of LDL S particles (nmol·L^{-1})	0.00152 (–2.12, 2.12)	–0.935 (–2.97, 1.1)
Concentration of LDL VS particles (nmol·L^{-1})	0.0136 (–1.02, 1.05)	–0.395 (–1.4, 0.612)
Concentration of HDL VL particles (nmol·L^{-1})	–1.49 (–5.26, 2.28)	0.373 (–3.21, 3.96)
Concentration of HDL L particles (nmol·L^{-1})	–21.4 (–51.7, 8.82)	–4.68 (–33.1, 23.7)
Concentration of HDL M particles (nmol·L^{-1})	–24.2 (–48.6, 0.172)	–15.6 (–39.4, 8.25)
Concentration of HDL S particles (nmol·L^{-1})	–6.7 (–28.9, 15.5)	–14.5 (–36.3, 7.24)
Concentration of HDL VS particles (nmol·L^{-1})	–3.18 (–16.9, 10.5)	–6.37 (–20, 7.27)
Total cholesterol concentration (mmol·L^{-1})	–0.0135 (–0.0424, 0.0154)	–0.0201 (–0.0488, 0.00865)
Cholesterol in CM particles (mmol·L^{-1})	0.000486 (0.000132, 0.00084)	0.000498 (0.000156, 0.000841)
Cholesterol in VLDL particles (mmol·L^{-1})	0.00856 (–0.00208, 0.0192)	0.00376 (–0.00646, 0.014)
Cholesterol in LDL particles (mmol·L^{-1})	–0.0118 (–0.033, 0.00945)	–0.0186 (–0.0395, 0.00222)
Cholesterol in HDL particles (mmol·L^{-1})	–0.0113 (–0.0219, –0.000717)	–0.00606 (–0.0161, 0.004)
Total triglycerides concentration (mmol·L^{-1})	0.0164 (0.000393, 0.0323)	0.0127 (–0.00147, 0.027)
Triglycerides in CM particles (mmol·L^{-1})	0.000908 (0.000166, 0.00165)	0.000783 (0.0000966, 0.00147)
Triglycerides in VLDL particles (mmol·L^{-1})	0.0143 (0.00264, 0.026)	0.00949 (–0.00205, 0.021)
Triglycerides in LDL particles (mmol·L^{-1})	0.00093 (–0.000504, 0.00236)	0.000418 (–0.000985, 0.00182)
Triglycerides in HDL particles (mmol·L^{-1})	0.00127 (–0.000285, 0.00283)	0.001 (–0.000555, 0.00255)
Mean diameter for VLDL particles (nm)	0.174 (0.0779, 0.271)	0.142 (0.0357, 0.248)

Mean diameter for LDL particles (nm)	–0.00782 (–0.0134, –0.00228)	–0.00605 (–0.0115, –0.000603)
Mean diameter for HDL particles (nm)	–0.00535 (–0.0141, 0.00341)	–0.000257 (–0.0084, 0.00789)

Each regression analysis was adjusted for monitor wear time, SES, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SES = socioeconomic status; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 4. Associations of 30 minutes' LPA and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.00251 (–0.0144, 0.00941)	–0.000866 (–0.01, 0.00828)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.00093 (–0.0537, 0.0519)	–0.0165 (–0.0654, 0.0324)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.0989 (–0.335, 0.137)	–0.098 (–0.31, 0.114)
Concentration of VLDL L3 particles (nmol·L^{-1})	–0.154 (–0.78, 0.473)	–0.123 (–0.716, 0.469)
Concentration of VLDL M particles (nmol·L^{-1})	–0.226 (–1.03, 0.577)	–0.195 (–0.97, 0.58)
Concentration of VLDL S particles (nmol·L^{-1})	0.286 (–0.402, 0.974)	0.298 (–0.386, 0.982)
Concentration of LDL L particles (nmol·L^{-1})	2.6 (–0.179, 5.39)	2.66 (–0.0992, 5.42)
Concentration of LDL M particles (nmol·L^{-1})	6.41 (0.176, 12.6)	6.61 (0.543, 12.7)
Concentration of LDL S particles (nmol·L^{-1})	2.61 (–0.448, 5.67)	2.74 (–0.19, 5.67)
Concentration of LDL VS particles (nmol·L^{-1})	1.11 (–0.387, 2.61)	1.17 (–0.281, 2.61)
Concentration of HDL VL particles (nmol·L^{-1})	–1.7 (–7.16, 3.75)	–1.96 (–7.13, 3.2)
Concentration of HDL L particles (nmol·L^{-1})	–8.25 (–52.1, 35.6)	–10.6 (–51.4, 30.3)
Concentration of HDL M particles (nmol·L^{-1})	–1.48 (–36.8, 33.8)	–2.71 (–37.1, 31.7)
Concentration of HDL S particles (nmol·L^{-1})	22.1 (–10, 54.2)	23.2 (–8.16, 54.5)
Concentration of HDL VS particles (nmol·L^{-1})	13.7 (–6.15, 33.5)	14.1 (–5.5, 33.7)
Total cholesterol concentration (mmol·L^{-1})	0.0322 (–0.00958, 0.074)	0.0331 (–0.00828, 0.0745)
Cholesterol in CM particles (mmol·L^{-1})	–0.000111 (–0.000701, 0.000478)	–0.000172 (–0.000684, 0.000339)
Cholesterol in VLDL particles (mmol·L^{-1})	0.000896 (–0.0145, 0.0163)	0.00157 (–0.0132, 0.0163)
Cholesterol in LDL particles (mmol·L^{-1})	0.0316 (0.000958, 0.0623)	0.0325 (0.00254, 0.0626)
Cholesterol in HDL particles (mmol·L^{-1})	0.00199 (–0.0133, 0.0173)	0.00126 (–0.0132, 0.0158)
Total triglycerides concentration (mmol·L^{-1})	–0.00279 (–0.0241, 0.0185)	–0.00351 (–0.0244, 0.0174)
Triglycerides in CM particles (mmol·L^{-1})	–0.0000225 (–0.00118, 0.00113)	–0.0005 (–0.00157, 0.000566)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.00468 (–0.0233, 0.014)	–0.0035 (–0.0185, 0.0115)
Triglycerides in LDL particles (mmol·L^{-1})	–0.000467 (–0.00254, 0.00161)	–0.000395 (–0.00241, 0.00162)
Triglycerides in HDL particles (mmol·L^{-1})	–0.000883 (–0.00314, 0.00137)	–0.000844 (–0.00308, 0.0014)
Mean diameter for VLDL particles (nm)	–0.0861 (–0.232, 0.06)	–0.0665 (–0.195, 0.0622)

Mean diameter for LDL particles (nm)	0.00247 (–0.00558, 0.0105)	0.00222 (–0.00565, 0.0101)
Mean diameter for HDL particles (nm)	–0.00456 (–0.0172, 0.00812)	–0.00527 (–0.017, 0.00646)

Each regression analysis was adjusted for monitor wear time, SES, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SES = socioeconomic status; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 5. Associations of 30 minutes' MVPA and lipoprotein measures in absolute concentration units, adjusted for sedentary time

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.0473 (–0.0688, –0.0259)	–0.0311 (–0.052, –0.0102)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.195 (–0.304, –0.0846)	–0.158 (–0.229, –0.0869)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.77 (–1.25, –0.294)	–0.627 (–1.01, –0.247)
Concentration of VLDL L3 particles (nmol·L^{-1})	–2.64 (–3.89, –1.38)	–1.72 (–2.93, –0.503)
Concentration of VLDL M particles (nmol·L^{-1})	–2.61 (–4.23, –0.992)	–1.67 (–3.26, –0.0791)
Concentration of VLDL S particles (nmol·L^{-1})	–0.113 (–1.51, 1.29)	0.298 (–1.11, 1.71)
Concentration of LDL L particles (nmol·L^{-1})	–2.07 (–7.72, 3.59)	–0.167 (–5.84, 5.51)
Concentration of LDL M particles (nmol·L^{-1})	–15.4 (–28, –2.75)	–8.64 (–21.1, 3.86)
Concentration of LDL S particles (nmol·L^{-1})	–10.7 (–16.9, –4.53)	–6.6 (–12.6, –0.573)
Concentration of LDL VS particles (nmol·L^{-1})	–4.63 (–7.66, –1.6)	–2.83 (–5.81, 0.146)
Concentration of HDL VL particles (nmol·L^{-1})	14.6 (3.59, 25.7)	6.27 (–4.36, 16.9)
Concentration of HDL L particles (nmol·L^{-1})	145 (56.3, 233)	70 (–13.9, 154)
Concentration of HDL M particles (nmol·L^{-1})	134 (62.7, 205)	97 (26.6, 167)
Concentration of HDL S particles (nmol·L^{-1})	–52.8 (–118, 12.5)	–17.5 (–82, 46.9)
Concentration of HDL VS particles (nmol·L^{-1})	–38.1 (–78.4, 2.17)	–24.2 (–64.6, 16.2)
Total cholesterol concentration (mmol·L^{-1})	–0.0611 (–0.146, 0.0238)	–0.0319 (–0.117, 0.0533)
Cholesterol in CM particles (mmol·L^{-1})	–0.00156 (–0.00266, –0.000454)	–0.00166 (–0.0026, –0.000714)
Cholesterol in VLDL particles (mmol·L^{-1})	–0.0484 (–0.0795, –0.0173)	–0.0272 (–0.0574, 0.00309)
Cholesterol in LDL particles (mmol·L^{-1})	–0.0681 (–0.13, –0.00585)	–0.0377 (–0.0995, 0.0241)
Cholesterol in HDL particles (mmol·L^{-1})	0.0508 (0.0199, 0.0818)	0.0276 (–0.00214, 0.0574)
Total triglycerides concentration (mmol·L^{-1})	–0.0657 (–0.109, –0.0224)	–0.0587 (–0.0965, –0.021)
Triglycerides in CM particles (mmol·L^{-1})	–0.0041 (–0.00646, –0.00174)	–0.00343 (–0.0055, –0.00136)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.0516 (–0.0987, –0.00454)	–0.0478 (–0.0772, –0.0185)
Triglycerides in LDL particles (mmol·L^{-1})	–0.00278 (–0.007, 0.00143)	–0.000456 (–0.00461, 0.0037)
Triglycerides in HDL particles (mmol·L^{-1})	–0.00298 (–0.00756, 0.00159)	–0.00179 (–0.0064, 0.00282)
Mean diameter for VLDL particles (nm)	–0.565 (–0.861, –0.27)	–0.501 (–0.775, –0.227)

Mean diameter for LDL particles (nm)	0.0307 (0.0146, 0.0469)	0.0232 (0.00716, 0.0393)
Mean diameter for HDL particles (nm)	0.0461 (0.0205, 0.0717)	0.0235 (–0.00065, 0.0476)

Each regression analysis was adjusted for monitor wear time, SES, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SES = socioeconomic status; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 6. Associations of 30 minutes' sedentary time and lipoprotein measures in absolute concentration units, adjusted for MVPA

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.0128 (–0.0239, –0.00162)	–0.00479 (–0.0155, 0.00591)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.04 (–0.097, 0.0169)	–0.0207 (–0.0572, 0.0158)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.0765 (–0.339, 0.186)	–0.0486 (–0.244, 0.146)
Concentration of VLDL L3 particles (nmol·L^{-1})	–0.405 (–1.06, 0.246)	–0.246 (–0.867, 0.375)
Concentration of VLDL M particles (nmol·L^{-1})	–0.342 (–1.18, 0.496)	–0.18 (–0.995, 0.635)
Concentration of VLDL S particles (nmol·L^{-1})	–0.244 (–0.969, 0.481)	–0.173 (–0.894, 0.548)
Concentration of LDL L particles (nmol·L^{-1})	–2.45 (–5.38, 0.482)	–2.12 (–5.03, 0.787)
Concentration of LDL M particles (nmol·L^{-1})	–8.1 (–14.7, –1.55)	–6.94 (–13.3, –0.531)
Concentration of LDL S particles (nmol·L^{-1})	–4.19 (–7.4, –0.983)	–3.48 (–6.57, –0.392)
Concentration of LDL VS particles (nmol·L^{-1})	–1.8 (–3.37, –0.225)	–1.49 (–3.01, 0.0393)
Concentration of HDL VL particles (nmol·L^{-1})	4.23 (–1.49, 9.96)	2.79 (–2.66, 8.24)
Concentration of HDL L particles (nmol·L^{-1})	35.2 (–10.6, 81.1)	22.3 (–20.7, 65.4)
Concentration of HDL M particles (nmol·L^{-1})	28.2 (–8.68, 65.1)	21.8 (–14.3, 57.9)
Concentration of HDL S particles (nmol·L^{-1})	–27.4 (–61.2, 6.48)	–21.3 (–54.3, 11.8)
Concentration of HDL VS particles (nmol·L^{-1})	–18.1 (–39, 2.79)	–15.7 (–36.4, 5.02)
Total cholesterol concentration (mmol·L^{-1})	–0.0374 (–0.0815, 0.00659)	–0.0324 (–0.0761, 0.0113)
Cholesterol in CM particles (mmol·L^{-1})	–0.000158 (–0.000729, 0.000414)	–0.000267 (–0.00075, 0.000216)
Cholesterol in VLDL particles (mmol·L^{-1})	–0.0104 (–0.0265, 0.00575)	–0.00672 (–0.0222, 0.00879)
Cholesterol in LDL particles (mmol·L^{-1})	–0.0384 (–0.0707, –0.00615)	–0.0332 (–0.0648, –0.0015)
Cholesterol in HDL particles (mmol·L^{-1})	0.0086 (–0.00743, 0.0246)	0.0046 (–0.0107, 0.0199)
Total triglycerides concentration (mmol·L^{-1})	–0.0108 (–0.0332, 0.0117)	–0.0112 (–0.0305, 0.00817)
Triglycerides in CM particles (mmol·L^{-1})	–0.000967 (–0.00219, 0.000255)	–0.000656 (–0.00172, 0.000405)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.0055 (–0.0299, 0.0189)	–0.00874 (–0.0238, 0.0063)
Triglycerides in LDL particles (mmol·L^{-1})	–0.00016 (–0.00234, 0.00202)	0.000241 (–0.00189, 0.00237)
Triglycerides in HDL particles (mmol·L^{-1})	0.000103 (–0.00227, 0.00247)	0.000309 (–0.00205, 0.00267)
Mean diameter for VLDL particles (nm)	–0.0604 (–0.214, 0.0928)	–0.0544 (–0.195, 0.0863)

Mean diameter for LDL particles (nm)	0.00421 (–0.00417, 0.0126)	0.00291 (–0.00532, 0.0112)
Mean diameter for HDL particles (nm)	0.0127 (–0.000557, 0.026)	0.00879 (–0.00356, 0.0211)

Each regression analysis was adjusted for monitor wear time, SES, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SES = socioeconomic status; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Table 7. Associations for an isotemporal substitution of 30 minutes' MVPA for 30 minutes' sedentary time and lipoprotein measures in absolute concentration units

Lipoprotein measure (units)	Coefficient (95% CI)	
	Not adjusted for adiposity	Adjusted for adiposity
Concentration of CM particles (nmol·L^{-1})	–0.0349 (–0.05, –0.0198)	–0.0263 (–0.0409, –0.0118)
Concentration of VLDL L1 particles (nmol·L^{-1})	–0.154 (–0.232, –0.0769)	–0.137 (–0.188, –0.0867)
Concentration of VLDL L2 particles (nmol·L^{-1})	–0.694 (–1.05, –0.339)	–0.577 (–0.841, –0.312)
Concentration of VLDL L3 particles (nmol·L^{-1})	–2.24 (–3.12, –1.36)	–1.47 (–2.32, –0.621)
Concentration of VLDL M particles (nmol·L^{-1})	–2.27 (–3.4, –1.14)	–1.49 (–2.61, –0.376)
Concentration of VLDL S particles (nmol·L^{-1})	0.127 (–0.849, 1.1)	0.469 (–0.518, 1.46)
Concentration of LDL L particles (nmol·L^{-1})	0.356 (–3.59, 4.3)	1.94 (–2.05, 5.92)
Concentration of LDL M particles (nmol·L^{-1})	–7.37 (–16.2, 1.46)	–1.76 (–10.5, 7.01)
Concentration of LDL S particles (nmol·L^{-1})	–6.57 (–10.9, –2.25)	–3.15 (–7.38, 1.08)
Concentration of LDL VS particles (nmol·L^{-1})	–2.85 (–4.96, –0.732)	–1.36 (–3.45, 0.734)
Concentration of HDL VL particles (nmol·L^{-1})	10.5 (2.75, 18.2)	3.51 (–3.95, 11)
Concentration of HDL L particles (nmol·L^{-1})	110 (48.3, 172)	47.9 (–11, 107)
Concentration of HDL M particles (nmol·L^{-1})	106 (56.2, 155)	75.2 (25.8, 125)
Concentration of HDL S particles (nmol·L^{-1})	–26 (–71.6, 19.5)	3.26 (–42, 48.5)
Concentration of HDL VS particles (nmol·L^{-1})	–20.4 (–48.5, 7.74)	–8.78 (–37.1, 19.6)
Total cholesterol concentration (mmol·L^{-1})	–0.024 (–0.0833, 0.0352)	0.000206 (–0.0595, 0.06)
Cholesterol in CM particles (mmol·L^{-1})	–0.00138 (–0.00213, –0.000617)	–0.00139 (–0.00206, –0.000729)
Cholesterol in VLDL particles (mmol·L^{-1})	–0.0381 (–0.0599, –0.0164)	–0.0205 (–0.0417, 0.000755)
Cholesterol in LDL particles (mmol·L^{-1})	–0.03 (–0.0735, 0.0134)	–0.00477 (–0.0481, 0.0386)
Cholesterol in HDL particles (mmol·L^{-1})	0.0423 (0.0208, 0.0639)	0.023 (0.00215, 0.0439)
Total triglycerides concentration (mmol·L^{-1})	–0.0543 (–0.0844, –0.0243)	–0.0476 (–0.0742, –0.0209)
Triglycerides in CM particles (mmol·L^{-1})	–0.00312 (–0.00477, –0.00147)	–0.00276 (–0.00421, –0.00132)
Triglycerides in VLDL particles (mmol·L^{-1})	–0.0459 (–0.0802, –0.0116)	–0.0392 (–0.0599, –0.0185)
Triglycerides in LDL particles (mmol·L^{-1})	–0.00265 (–0.00559, 0.000294)	–0.000711 (–0.00363, 0.00221)
Triglycerides in HDL particles (mmol·L^{-1})	–0.00309 (–0.00628, 0.0000993)	–0.0021 (–0.00533, 0.00113)
Mean diameter for VLDL particles (nm)	–0.504 (–0.712, –0.297)	–0.446 (–0.637, –0.254)

Mean diameter for LDL particles (nm)	0.0266 (0.0153, 0.0379)	0.0203 (0.00907, 0.0316)
Mean diameter for HDL particles (nm)	0.0336 (0.0158, 0.0515)	0.0148 (–0.00214, 0.0317)

Each regression analysis was adjusted for monitor wear time, SES, sex, and sexual maturity.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SES = socioeconomic status; VLDL = very low-density lipoprotein; L = large; M = medium; S = small; VS = very small; VL = very large; C = cholesterol; TG = triglycerides.

Paper II

Prospective associations between aerobic fitness and lipoprotein subclasses in a cohort of Norwegian schoolchildren.

Abstract

Background: 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 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 profile.

Results: Higher levels of aerobic fitness were associated with a favourable lipoprotein 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; p <0.001 for VLDL cholesterol concentration). In the prospective analysis, the favourable pattern of associations persisted, though associations with the VLDL classes tended to be more consistent with the cross-sectional associations than those of low-density lipoproteins and high-density lipoproteins. Additional adjustment for adiposity attenuated the associations in both cross-sectional and prospective models. Nevertheless, an independent effect of aerobic fitness remained for some measures.

Conclusion: 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] 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 load.[4] Nor can these measures demonstrate divergent directions of associations among lipoprotein subclasses, such as those observed for high-density lipoprotein (HDL) with long-term leisure-time physical activity,[5] adiposity,[6] and risk of ischaemic stroke.[7] By providing a more nuanced description, metabolic profiling of the lipidome—lipidomics—reveals more of the complexity of biological processes and interactions with environmental and lifestyle exposures, and hence can generate novel insights and hypotheses.

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

2. Materials and methods

Additional information on the 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 seven-month cluster randomised controlled trial that investigated the effect of a school-based physical activity intervention on academic performance of fifth-grade schoolchildren in Sogn og 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. We found both intervention and control groups to be equally active and fit at baseline and follow-up, hence we pooled both groups together for the present cohort study. 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 $\dot{V}O_2$.[10]

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.

NMR 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). The NMR spectral regions were selected as explanatory variables to partial least squares (PLS) modelling. Responses in the PLS models included a number of measures of the four major lipoprotein classes; chylomicron (CM), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL); total cholesterol concentration, and total triglycerides concentration, determined from high-performance liquid chromatography (HPLC).[11] In addition, we calculated non-HDL cholesterol concentration by subtracting the HDL cholesterol concentration from total cholesterol concentration. One hundred and six serum samples were randomly selected to be analysed by both NMR and HPLC and used for PLS modelling. Individual PLS models with optimal prediction ability were calculated using a Monte Carlo resampling approach.[12] In total, we calculated 57 variables for subsequent analysis (Supplementary Material Table 1). 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, is retained. 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.

2.4. Anthropometry

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 ($kg \cdot m^{-2}$). Waist circumference was measured twice to the nearest 0.1cm 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.[13] The children's ages at the time of testing were rounded down to the nearest half-year to provide conservative prevalence estimates.

2.5. Sexual maturity

The children self-reported their sexual maturity with reference to a standard set of colour images[14] with accompanying descriptions corresponding to the Tanner staging method. 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).

2.6. 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. Each parent or guardian completed a self-report questionnaire designed for the ASK study, choosing between six categories of education completed. The four lower categories were combined given the low frequencies of respondents in these groups (n = 4, 15, 193, and 137 for categories 1–4, respectively of 1069 children with valid data). The three categories used in the present analysis were: i) upper secondary school, ii) less than four years of college/university, iii) equal to or more than four years of college/university.

2.7. Statistical approach

We examined cross-sectional associations between aerobic fitness and 57 lipoprotein measures at baseline using multiple linear regression, adjusting for age, parents' education, sex, and sexual maturity (Model 1). Cluster-robust standard errors were calculated for each model to account for potential within-cluster correlation of the school variable and to obviate transforming skewed outcome measures.[15] We further examined these associations adjusted for waist circumference as a measure of adiposity (Model 2). For the prospective analyses, we regressed follow-up values of the lipoprotein measures on both the Model 1 and 2 predictors, and additionally adjusted for baseline measures of the respective lipoprotein measure. Our analytical sample included those children that had complete data for all variables included in the respective models, hence can differ.

As supplementary analyses, we substituted BMI for waist circumference as our measure of adiposity in the cross-sectional and prospective models. To compare the magnitudes of main effects of aerobic fitness and waist circumference, we performed analyses between waist circumference and the lipoprotein profile, adjusting for the same confounders as in Model 1. In the prospective model, we additionally adjusted for baseline measures of the respective lipoprotein variables. We also examined the association between waist circumference and aerobic fitness, adjusting for the Model 1 confounders and additionally for baseline aerobic fitness in the prospective model.

We report effect size, 95% confidence interval (CI), and p value for all lipoprotein measures in each regression analysis. Aerobic fitness and all lipoprotein measures were standardised (mean = 0.0; SD = 1.0) prior to regression. When modelled as the primary exposure, waist circumference was also standardised. Hence, regression coefficients represent the SD unit change in lipoprotein measure for a 1 SD increase in aerobic fitness or waist circumference. We used principal component analysis (PCA) to estimate the effective number of independent tests to use for multiple testing correction. The rationale for this method has been described previously and applied in a number of metabolic profile studies.[16–18] Using standardised values of the 57 baseline lipoprotein measures, we calculated that 4 principal components explained >95% of the variance. Hence, our Bonferroni-corrected threshold for assessing associations is 0.05/5 = 0.01 (i.e., p <0.01). We performed all statistical analyses in R version 3.6.3.[19] 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 PCA analysis with **factoextra** (1.0.6) and 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

The analytical sample comprised 858 children (49.0% girls) with complete data (Supplementary Material Figure 1). The average interval between baseline and follow-up testing was 34.3 weeks. Those children excluded due to missing data (n = 271) were similar to our analytical sample (Table 1). Of the 858 children with complete data for the cross-sectional analysis, 782 had valid blood measurements at follow-up and were eligible for inclusion in the prospective analysis.

3.2. Cross-sectional associations

Aerobic fitness was associated with almost all lipoprotein measures at baseline (Figure 1). Per SD unit (103 m) increase in distance run for the Andersen test, there were inverse associations with all measures of the apolipoprotein B-containing (ApoB-containing) lipoprotein subclasses (e.g., $–2.61 \times 10^{–1}$ nmol·L$^{–1}$ or –0.21 SD; 95% CI = –0.30, –0.13; p <0.001 for VLDL L1 subclass particle number). These tended to be more modest for the VLDL S and LDL L subclasses, especially the particle numbers and cholesterol concentrations. There were also inverse associations with particle numbers and cholesterol concentrations of the smaller HDL particles, total and non-HDL cholesterol concentration, total and HDL triglycerides concentration, and average VLDL particle diameter (e.g., $–4.26 \times 10^{–3}$ mmol·L$^{–1}$ or –0.12 SD; 95% CI = –0.19, –0.04; p = 0.004 for HDL triglycerides concentration). Despite aerobic fitness being positively associated with the particle number and cholesterol concentration of the HDL M subclass, the association was inverse with the triglycerides concentration. There were positive associations with the particle numbers and cholesterol concentrations of the larger HDL particles, total HDL cholesterol concentration, and the average particle diameters of HDL and LDL particles ($5.11 \times 10^{–2}$ nm or 0.24 SD units; 95% CI = 0.17, 0.31; p <0.001 for HDL particles). The association with the triglycerides concentration of the HDL VL subclass was negligible.

The associations for all but one measure (HDL VL triglycerides concentration) were attenuated in the model adjusted for waist circumference (Figure 2). The degree of attenuation varied between classes, within classes, and between different measures within the same subclass. This was most pronounced for the HDL subclasses. For example, the greatest attenuation for an HDL subclass was 0.15 SD, whereas for an LDL subclass it was 0.10 SD. The associations with the average particle size of the VLDL, LDL, and HDL classes were attenuated substantially (absolute difference range = 0.09–0.15 SD). All association directions remained the same.

3.3. Prospective associations

In the prospective analysis, the association directions between an SD unit (102 m) increment in aerobic fitness remained the same as those in the cross-sectional model for all 57 measures (Figure 3). Magnitudes were attenuated for all but one measure (HDL VL triglycerides concentration), but the degree of attenuation varied greatly (absolute difference range = 0.03–

0.23 SD). Comparing the cross-sectional and prospective models, the associations with the VLDL subclasses were typically more consistent than the measures of LDL and HDL subclasses (e.g., –1.73 x 10^{-1} nmol·L^{-1} or –0.17 SD; 95% CI = –0.26, –0.08; p <0.001 for VLDL L1 subclass particle number). Exceptions include the triglycerides concentrations of the smaller LDL and HDL subclasses. In general, the associations of aerobic fitness with measures of subclass triglycerides concentrations tended to be more consistent than with particle numbers or cholesterol concentrations. The range (absolute difference between cross-sectional and prospective models) for triglycerides concentrations across all subclasses was 0.03–0.12 SD, compared to 0.03–0.22 SD for cholesterol concentrations. There was substantial variation in the consistency of associations for average particle diameters (absolute difference range = 0.04–0.23 SD).

Having adjusted for waist circumference, the pattern of associations was broadly similar to the unadjusted prospective model (Figure 4). Association directions were the same for all measures of ApoB-containing lipoproteins, though the magnitudes were attenuated for each (absolute difference range = 0.01–0.09 SD). The association directions changed for some measures of the larger HDL subclasses and also average HDL particle diameter, though the effect sizes were small before and after adjustment for waist circumference. The associations were generally more modest than in the other models but tended to be stronger for the ApoB-containing particles (e.g., –7.74 x 10^{-2} nmol·L^{-1} or –0.08 SD; 95% CI = –0.15, 0.00; p = 0.06 for VLDL L1 subclass particle number).

3.4. Supplementary analyses

Using BMI instead of waist circumference as our measure of adiposity had a negligible effect on either the pattern of associations or association magnitudes in both the cross-sectional and prospective models (Supplementary Material Tables 4 and 9).

In the cross-sectional analysis of waist circumference and lipoprotein profile (n = 894 with complete data), the association magnitudes were greater compared to those in the analysis of Model 1 for all measures except the VLDL S and LDL L cholesterol concentrations (Supplementary Material Figure 2). The association directions were opposite for all measures except HDL VL triglycerides concentration (e.g., 3.56 x 10^{-1} nmol·L^{-1} or 0.29 SD; 95% CI = 0.18, 0.36; p <0.001 for VLDL L1 subclass particle number). The same was true in the prospective analysis (n = 807 with complete data), although the association between aerobic fitness and total cholesterol concentration was marginally stronger than between waist circumference and total cholesterol concentration (Supplementary Material Figure 3).

Larger waist circumference 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). The sample size for the prospective analysis was 737 as some children did not have aerobic fitness measured at follow-up.

The results of each analysis with outcome variables in absolute units are provided in Supplementary Material Tables 2–11.

4. Discussion

We used targeted metabolomics to investigate cross-sectional and prospective associations between aerobic fitness and lipoprotein 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 CM and VLDL particles, and with measures of subclass triglycerides concentration, in the prospective model, which may indicate a greater influence 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.[20] Compared to aerobic fitness, the associations between an 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. Still, adjustment for waist circumference as a confounder did not completely eliminate the associations and suggests that some influence of aerobic fitness is independent of adiposity.

To our knowledge, this is the first study to investigate prospective associations between aerobic fitness and a comprehensive lipoprotein profile in children. A recent study in adults examined associations of the serum metabolome between high and low aerobic fitness individuals and found a favourable pattern of associations in more fit individuals.[21] Another explored prospective associations of accelerometer-measured physical activity with lipoprotein profiles in adolescents.[18] In their analysis of moderate- to vigorous-intensity physical activity (MVPA) mutually adjusted for time spent sedentary, the authors also reported a beneficial pattern of associations. Having additionally adjusted for total fat mass (measured using dual-energy X-ray absorptiometry), the associations were modestly attenuated, which suggests that confounding by adiposity did not fully explain their results. In our analyses, attenuation by adiposity appears more marked, which possibly reflects our less precise measure of adiposity or the influence of body size on aerobic performance tests.

Vigorous physical activity may improve aerobic fitness[22] and prevent excessive adiposity[23] in children and youth. However, the associations between physical activity and adiposity are likely bidirectional and tend to be stronger when adiposity is modelled as the exposure.[24] Adiposity also has a strong, causal effect on lipoprotein metabolism.[6] Reducing adiposity therefore, whether through increased physical activity or reduced energy intake, should be a priority. Not only would this benefit lipoprotein metabolism directly, but also through the independent metabolic effects of higher aerobic fitness resulting from increased physical activity (if of sufficient intensity).

Strengths of our study include the detailed measures of lipoprotein metabolism quantified using a targeted NMR metabolomics platform, which enabled the investigation of a number of different lipoprotein measures, including divergent associations of HDL particle subclasses. We included prospective analyses, investigating associations of aerobic fitness with lipoprotein measures over time, including adjustment for the respective lipoprotein measurement at

baseline. We had a relatively large sample size given the population and measures analysed, and high compliance of the children with both the fitness testing and blood sampling. 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. 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.[25] 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 profiles, they are static snapshots of dynamic processes so can only speculate as to any mechanistic explanations for these associations. Though we adjusted for a number of variables that we expect to influence metabolic health, 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 $\dot{V}O_2$. 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 $\dot{V}O_2$ by indirect calorimetry.[26] However, previous studies on a smaller but similar cohort showed significantly greater impact of BMI on peak $\dot{V}O2$ than on performance in the Andersen test, and stronger associations with lipoprotein measures for the Andersen test than for peak $\dot{V}O2$.[27,28] Still, these results should be interpreted carefully due to the relatively small sample size and different analytical approach used.

4.1. Conclusion

In conclusion, higher levels of aerobic fitness are prospectively associated with a favourable lipoprotein 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.

References

1 Shah RV, Murthy VL, Colangelo LA, *et al.* Association of Fitness in Young Adulthood With Survival and Cardiovascular Risk: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *JAMA Intern Med* 2016; **176**: 87–95.

2 Anderssen SA, Cooper AR, Riddoch C, *et al.* Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 526–31.

3 Kelley GA, Kelley KS. Aerobic exercise and lipids and lipoproteins in children and adolescents: A meta-analysis of randomized controlled trials. *Atherosclerosis* 2007; **191**: 447–53.

4 Ala-Korpela M. The culprit is the carrier, not the loads: cholesterol, triglycerides and apolipoprotein B in atherosclerosis and coronary heart disease. *Int J Epidemiol* 2019; **48**: 1389–92.

5 Kujala UM, Makinen V-P, Heinonen I, *et al.* Long-term Leisure-time Physical Activity and Serum Metabolome. *Circulation* 2013; **127**: 340–8.

6 Würtz P, Wang Q, Kangas AJ, *et al.* Metabolic Signatures of Adiposity in Young Adults: Mendelian Randomization Analysis and Effects of Weight Change. *PLoS Med* 2014; **11**: e1001765.

7 Holmes MV, Millwood IY, Kartsonaki C, *et al.* Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *J Am Coll Cardiol* 2018; **71**: 620–32.

8 Resaland GK, Aadland E, Moe VF, *et al.* Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. *Prev Med* 2016; **91**: 322–8.

9 World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* 2013; **310**: 2191–4.

10 Aadland E, Terum T, Mamen A, Andersen LB, Resaland GK. The Andersen Aerobic Fitness Test: Reliability and Validity in 10-Year-Old Children. *PLOS ONE* 2014; **9**: e110492.

11 Okazaki M, Usui S, Ishigami M, *et al.* Identification of unique lipoprotein subclasses for visceral obesity by component analysis of cholesterol profile in high-performance liquid chromatography. *Arterioscler Thromb Vasc Biol* 2005; **25**: 578–84.

12 Kvalheim OM, Arneberg R, Grung B, Rajalathi T. Determination of optimum number of components in partial least squares regression from distributions of the root-mean-squared error obtained by Monte Carlo resampling. *J Chemom* 2018; **32**: e2293.

13 Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–94.

14 Carel J-C, Léger J. Precocious Puberty. *N Engl J Med* 2008; **358**: 12.

15 Zeileis A. Econometric Computing with HC and HAC Covariance Matrix Estimators. *J Stat Softw* 2004; **11**. DOI:10.18637/jss.v011.i10.

16 Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008; **32**: 361–9.

17 Ferreira DLS, Williams DM, Kangas AJ, *et al.* Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. *PLOS Med* 2017; **14**: e1002376.
18 Bell JA, Hamer M, Richmond RC, Timpson NJ, Carslake D, Smith GD. Associations of device-measured physical activity across adolescence with metabolic traits: Prospective cohort study. *PLOS Med* 2018; **15**: e1002649.
19 R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020 https://www.R-project.org/.
20 Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med* 2011; **17**: 594–603.
21 Kujala UM, Vaara JP, Kainulainen H, Vasankari T, Vaara E, Kyröläinen H. Associations of Aerobic Fitness and Maximal Muscular Strength With Metabolites in Young Men. *JAMA Netw Open* 2019; **2**: e198265–e198265.
22 Carson V, Rinaldi RL, Torrance B, *et al.* Vigorous physical activity and longitudinal associations with cardiometabolic risk factors in youth. *Int J Obes* 2014; **38**: 16–21.
23 Hamer M, Stamatakis E. Relative proportion of vigorous physical activity, total volume of moderate to vigorous activity, and body mass index in youth: the Millennium Cohort Study. *Int J Obes* 2018; **42**: 1239–42.
24 Richmond RC, Davey Smith G, Ness AR, den Hoed M, McMahon G, Timpson NJ. Assessing Causality in the Association between Child Adiposity and Physical Activity Levels: A Mendelian Randomization Analysis. *PLoS Med* 2014; **11**: e1001618.
25 WHO Regional Office for Europe. WHO European Childhood Obesity Surveillance Initiative: overweight and obesity among 6–9-year-old children. Report of the third round of data collection 2012–2013. Copenhagen: World Health Organization, 2018.
26 Armstrong N, Welsman J. Clarity and Confusion in the Development of Youth Aerobic Fitness. *Front Physiol* 2019; **10**: 979.
27 Resaland GK, Rajalahti T, Aadland E, Kvalheim OM. Strong association between cardiorespiratory fitness and serum lipoprotein subclass pattern in prepubertal healthy children. *Scand J Med Sci Sports* 2018; **28**: 220–7.
28 Aadland E, Kvalheim OM, Rajalahti T, Skrede T, Resaland GK. Aerobic fitness and metabolic health in children: A clinical validation of directly measured maximal oxygen consumption versus performance measures as markers of health. *Prev Med Rep* 2017; **7**: 74–6.

Conflict of interest
The Authors declare that there is no conflict of interest.

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Authors' 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.

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Figure legends

Figure 1. Cross-sectional associations of aerobic fitness with 57 lipoprotein measures

The associations were adjusted for age, parents' 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.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 2. Cross-sectional associations of aerobic fitness with 57 lipoprotein measures, adjusted for waist circumference

The associations were adjusted for age, parents' 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.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 3. Prospective associations of aerobic fitness with 57 lipoprotein measures

The associations were adjusted for age, parents' education, sex, sexual maturity, and baseline lipoprotein measure. 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.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 4. Prospective associations of aerobic fitness with 57 lipoprotein measures, adjusted for waist circumference

The associations were adjusted for age, parents' education, sex, sexual maturity, baseline lipoprotein measure, 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.

Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

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

Characteristic	Analytical sample (n = 858)		Excluded cases (n = 271)		Difference
	n (%)	Mean (SD)	Data available – n (%)	Mean (SD)	p value[a]
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 (8.1)	237	37.1 (8.1)	0.888
Body mass index ($kg \cdot m^{-2}$)[c]		18 (3.0)	237	18.2 (3.1)	0.333
≥25	189 (22.0)		46 (19.4)		0.075
≥30	37 (4.3)		8 (3.4)		
Waist circumference (cm)		61.9 (7.4)	236	62.3 (7.6)	0.456
Parents' education[b]					
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[c]					
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
Lipid profile (baseline)					
TC ($mmol \cdot L^{-1}$)		4.5 (0.7)	150	4.4 (0.6)	0.390
LDL-C ($mmol \cdot L^{-1}$)		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})[d]		0.7 [0.5, 0.9]	150	0.7 [0.5, 0.9]	0.696[e]
Lipid profile (follow-up)	782				
TC (mmol·L^{-1})		4.5 (0.6)	159	4.5 (0.6)	0.941
LDL-C (mmol·L^{-1})		2.6 (0.6)	159	2.6 (0.6)	0.939
HDL-C (mmol·L^{-1})		1.6 (0.3)	159	1.6 (0.3)	0.426
TG (mmol·L^{-1})[d]		0.7 [0.5, 0.9]	159	0.7 [0.5, 0.9]	0.606[e]

[a]Continuous characteristics compared using linear regression, categorical characteristics using binomial logistic regression.
[b]For comparison, dichotomised to 'Upper secondary school' and 'Higher than upper secondary school'.
[c]For comparison, dichotomised to Tanner stage 1 and Tanner stage ≥2.
[d]Median [IQR].
[e]Comparison performed using *log*(TG).
Abbreviations: ASK = Active Smarter Kids; BMI = body mass index; HDL-C = high-density lipoprotein; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides.

Figure 1.

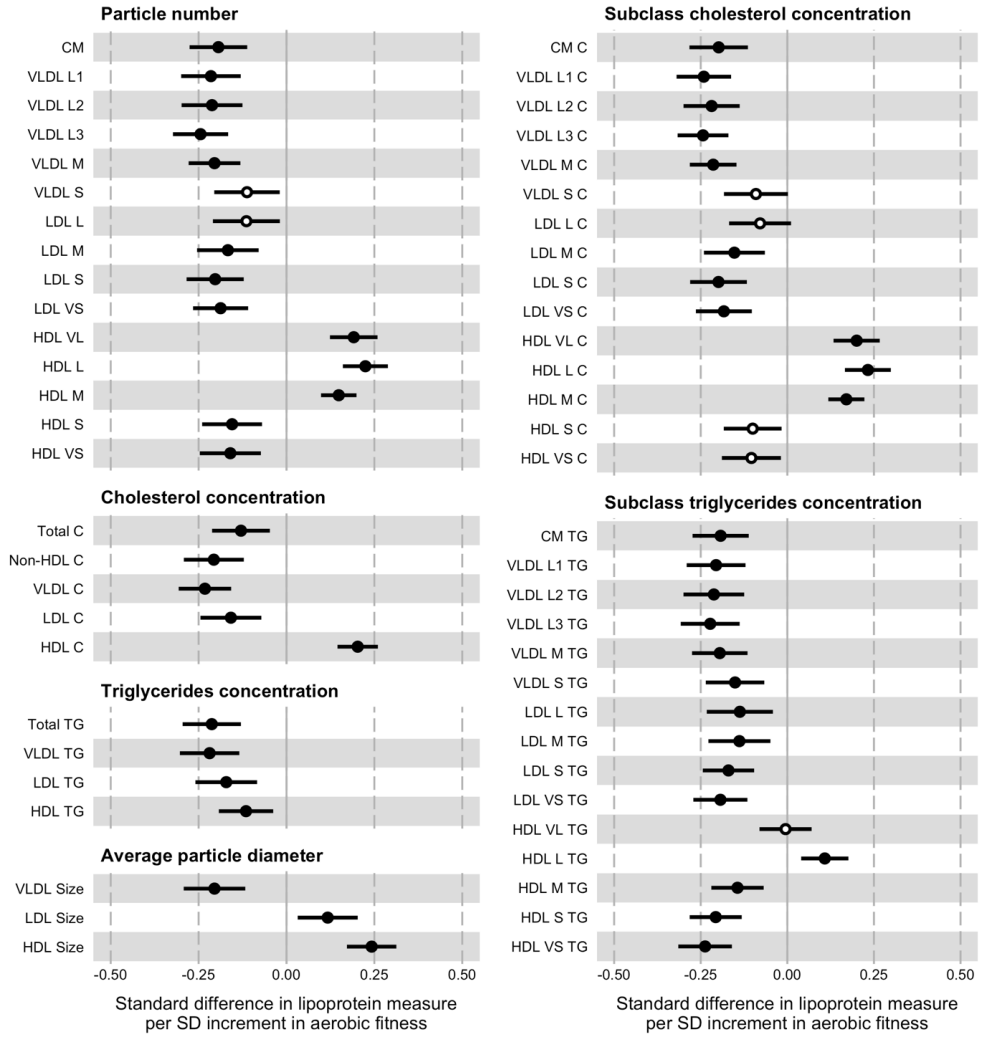

Particle number
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
HDL S TG
HDL VS TG
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness

Figure 2.

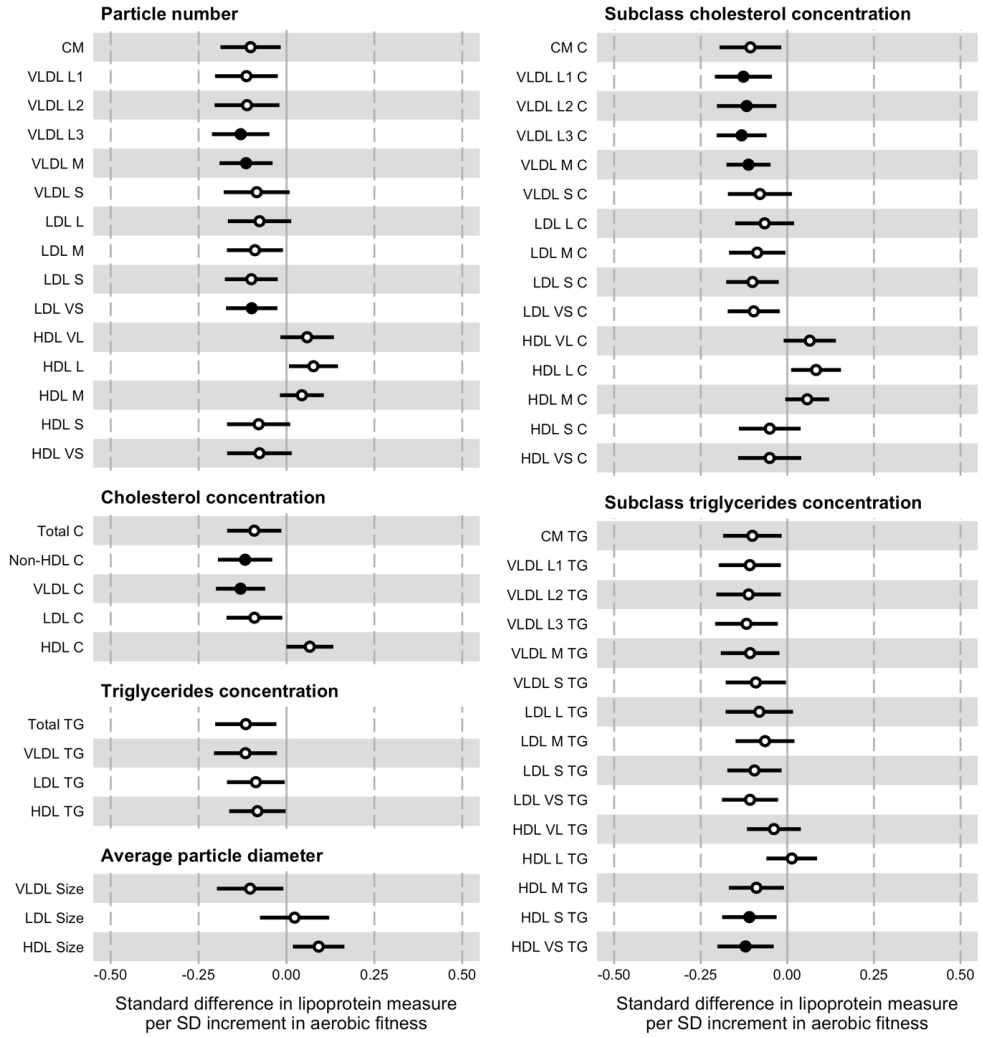
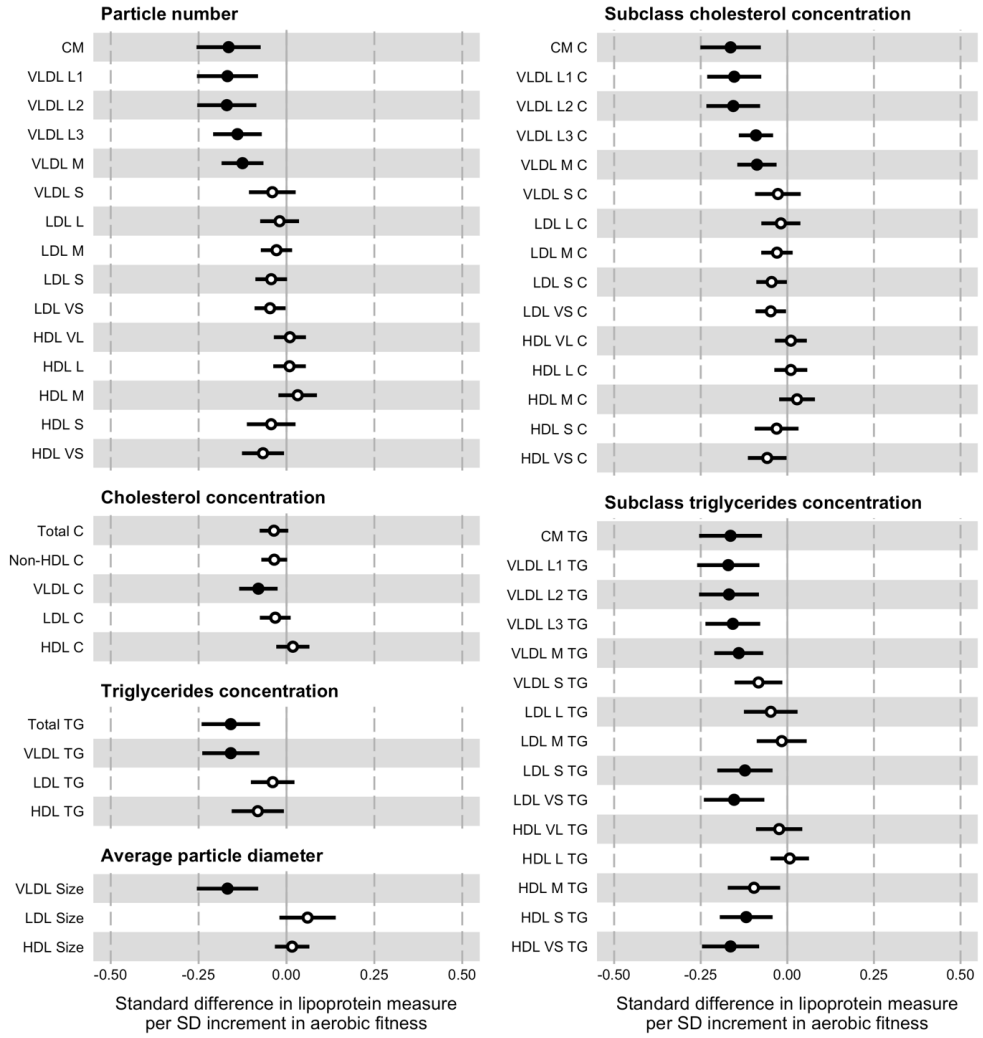

Particle number
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
HDL S TG
HDL VS TG
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness

Figure 3.


Particle number
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
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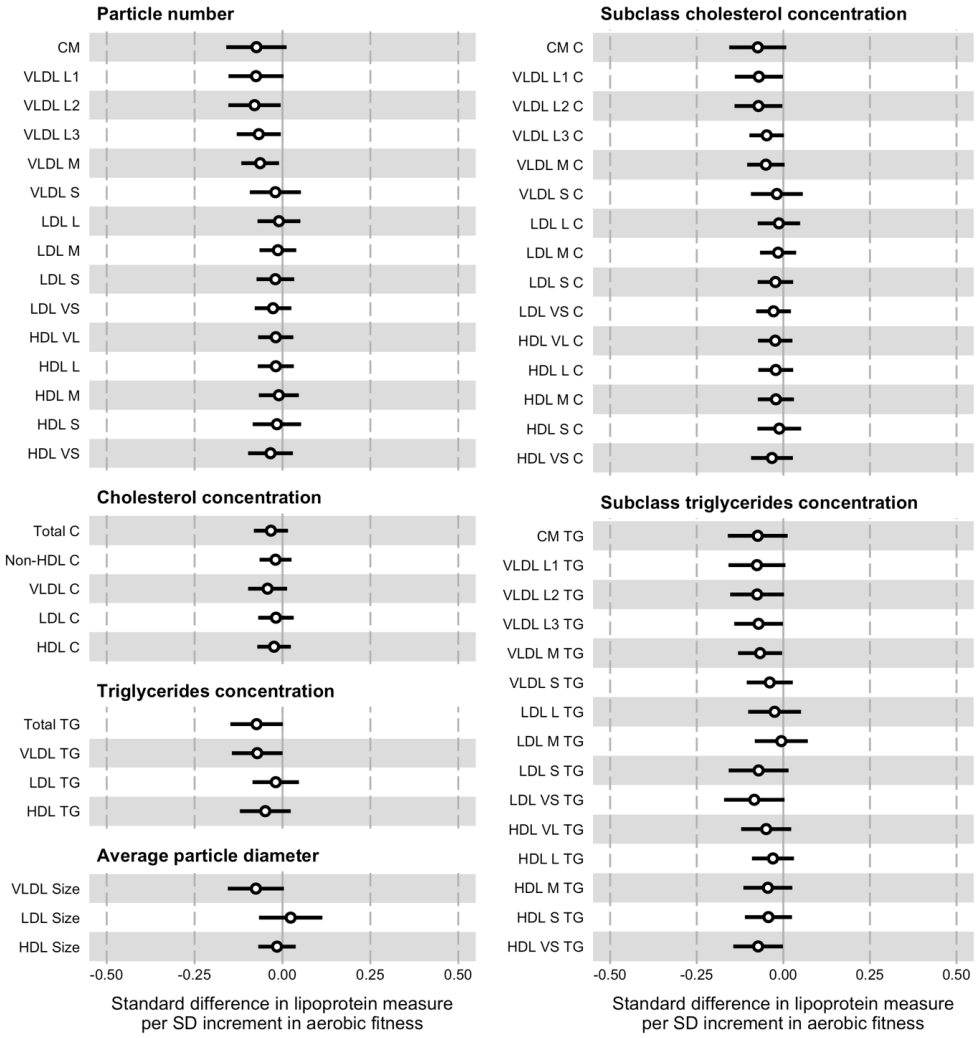
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
HDL S TG
HDL VS TG
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness

Figure 4.


Particle number
CM
VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
HDL S TG
HDL VS TG
-0.50
-0.25
0.00
0.25
0.50
Standard difference in lipoprotein measure per SD increment in aerobic fitness

Paper II supplementary material

Supplementary methods

Assessment of aerobic fitness

Following an explanation of the test and five-minute warm-up, the children ran back and forth between two parallel lines set 20 metres apart for a total of ten minutes, alternately running for 15 seconds then pausing for 15 seconds. Each time they reached either of the two end lines, they had to touch beyond the line with one finger before they could run back in the opposite direction. The objective was to cover as great a distance as possible within the time permitted. Study research assistants supervised testing and recorded the total distance (m) covered for each child. In our study, we used running distance as the main exposure as opposed to applying a prediction equation to convert distance covered to an estimate of peak $\dot{V}O_2$.

Blood samples

Serum was drawn from an antecubital vein and obtained according to a standardised protocol consisting of the following steps: i) Blood plasma was collected in 5 ml VACUETTE Serum Gel with Activator blood collection tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria). ii) The tubes were carefully inverted five times and placed vertically for coagulation. iii) After 30 minutes, the samples were centrifuged at 2000 G for ten minutes. Serum was then visually inspected for residue and centrifugation was repeated if residue was present. iv) The tubes were kept in a refrigerator at 4°C before pipetting 0.5 ml into cryo tubes. v) The cryo tubes were then stored in a freezer at –20°C for up to 2 days before finally being stored at –80°C until analysis. The frozen serum samples were thawed at room temperature for approximately one hour. Aliquots of 120 µl were carefully mixed with equal amounts of phosphate buffer in Eppendorf tubes, and transferred to 3 mm SampleJet tubes by syringe. A fill height of 4 cm was used amounting to approximately 180 µl.

^{1}H NMR protocol

Serum spectra were recorded at 310 K, using a one-dimensional NOESY (noesygppr1d) pulse sequence. A total of 32 scans were acquired, using 96k data points and 30 ppm spectral width. The spectra were processed with 0.3 Hz line broadening, automatically phase-corrected and aligned to the lactate signal at 1.32 ppm. Spectra were normalised to an ERETIC signal, functioning as an external reference. Details of the ^{1}H NMR protocol have been described previously.[1]

PLS modelling

Response variables for PLS modelling comprised: i) 20 subclasses for particle number, which we reduced to 15, ii) average particle size of VLDL, LDL, and HDL classes, iii) cholesterol and triglycerides concentrations of CM, VLDL, LDL, and HDL classes, iv) total triglycerides concentration, and total cholesterol (TC) concentration. Due to the elution of lipid-poor pre-β1 HDL,[2] the "spherical particle model" cannot be applied to calculate the particle number for the HDL7 minor subclass.[3] Hence, the particle number of very small HDL was calculated from HDL6 only. In addition, we calculated non-HDL cholesterol concentration by subtracting the HDL cholesterol concentration from total cholesterol concentration.

References

1 Jones PR, Rajalahti T, Resaland GK, *et al.* Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis* 2019; **288**: 186–93.

2 Nanjee MN, Brinton EA. Very Small Apolipoprotein A-I-containing Particles from Human Plasma: Isolation and Quantification by High-Performance Size-Exclusion Chromatography. *Clin Chem* 2000; **46**: 207–23.

3 Okazaki M, Yamashita S. Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using "Spherical Particle Model". *J Oleo Sci* 2016; **65**: 265–82.

Figure 1. Flow of study participants

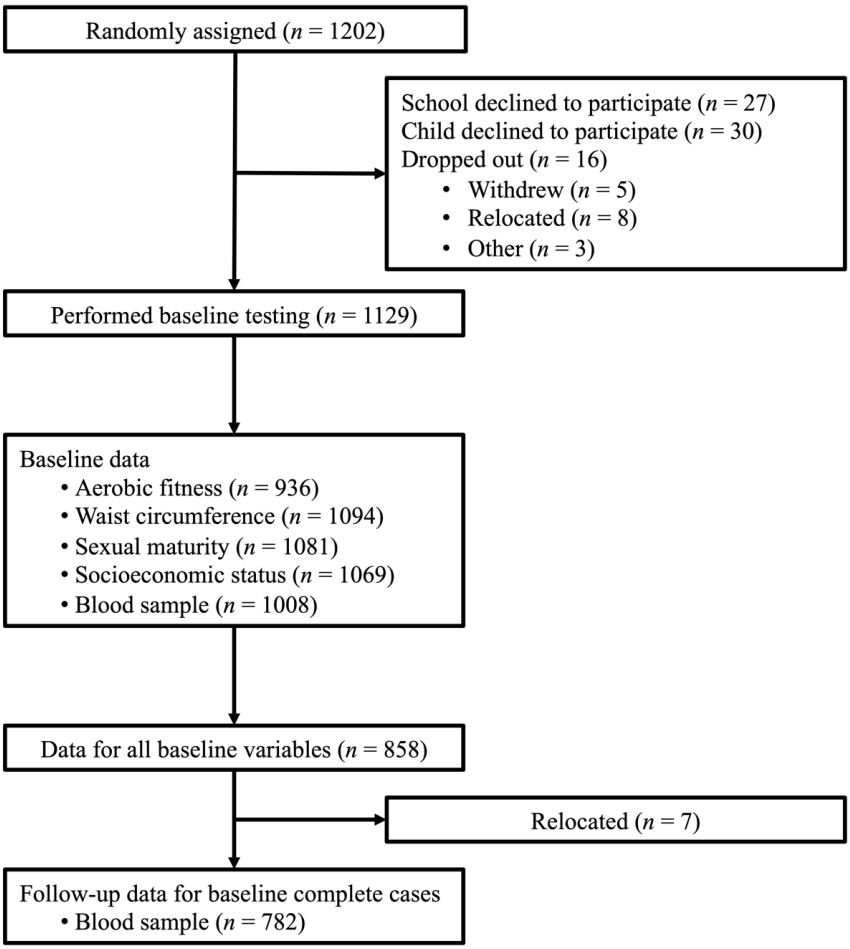


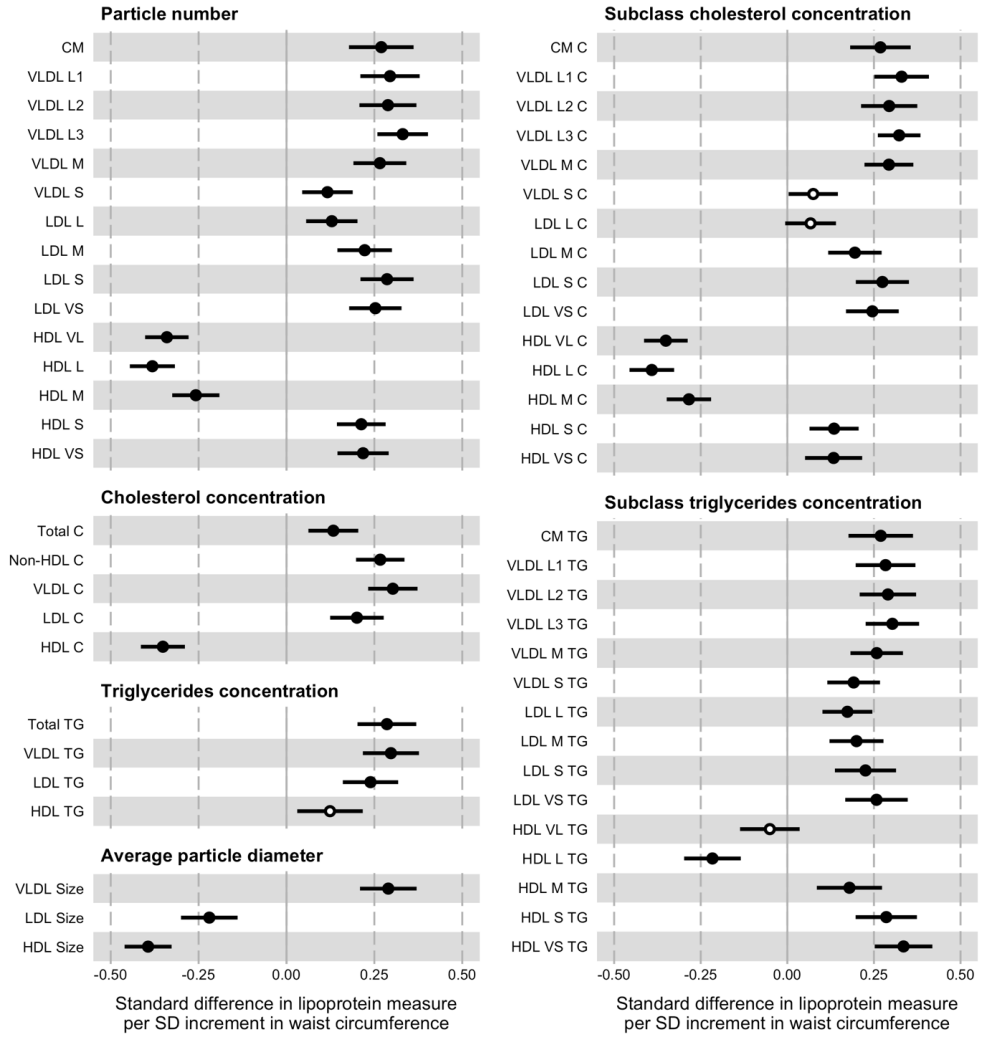
Table 1. Mean and standard deviation (SD) for each lipoprotein measure in absolute concentration units, measured at baseline

Lipoprotein measure	Baseline (n = 858)	Follow-up (n = 782)
	Mean (SD)	Mean (SD)
CM PN (nmol·L^{-1})	0.237 (0.374)	0.223 (0.303)
VLDL L1 PN (nmol·L^{-1})	1.017 (1.214)	0.964 (1.027)
VLDL L2 PN (nmol·L^{-1})	4.299 (4.482)	4.049 (3.879)
VLDL L3 PN (nmol·L^{-1})	19.183 (10.580)	18.567 (9.999)
VLDL M PN (nmol·L^{-1})	29.121 (13.485)	28.963 (12.869)
VLDL S PN (nmol·L^{-1})	45.139 (11.395)	45.783 (11.274)
LDL L PN (nmol·L^{-1})	213.495 (46.340)	217.031 (44.262)
LDL M PN (nmol·L^{-1})	469.827 (103.730)	473.113 (99.475)
LDL S PN (nmol·L^{-1})	222.833 (50.938)	222.166 (49.548)
LDL VS PN (nmol·1^{-1})	0.176 (0.037)	0.176 (0.036)
HDL VL PN (nmol·L^{-1})	0.086 (0.034)	0.090 (0.036)
HDL L PN (nmol·L^{-1})	1630.760 (727.301)	1716.415 (749.196)
HDL M PN (nmol·L^{-1})	4318.116 (595.988)	4333.659 (582.385)
HDL S PN (nmol·L^{-1})	5237.059 (536.111)	5172.011 (564.153)
HDL VS PN (nmol·L^{-1})	2675.859 (246.142)	2642.924 (252.081)
CM C (mmol·L^{-1})	0.010 (0.014)	0.010 (0.011)
VLDL C (mmol·L^{-1})	0.642 (0.258)	0.643 (0.242)
VLDL L1 C (mmol·L^{-1})	0.020 (0.017)	0.019 (0.015)
VLDL L2 C (mmol·L^{-1})	0.041 (0.041)	0.040 (0.036)
VLDL L3 C (mmol·L^{-1})	0.205 (0.089)	0.201 (0.088)
VLDL M C (mmol·L^{-1})	0.164 (0.079)	0.163 (0.072)
VLDL S C (mmol·L^{-1})	0.208 (0.055)	0.213 (0.054)
LDL C (mmol·L^{-1})	2.236 (0.510)	2.258 (0.490)
LDL L C (mmol·L^{-1})	0.718 (0.169)	0.732 (0.161)
LDL M C (mmol·L^{-1})	0.969 (0.229)	0.976 (0.222)
LDL S C (mmol·L^{-1})	0.389 (0.095)	0.389 (0.093)
LDL VS C (mmol·L^{-1})	0.165 (0.036)	0.165 (0.035)
HDL C (mmol·L^{-1})	1.463 (0.256)	1.484 (0.253)
HDL VL C (mmol·L^{-1})	0.080 (0.033)	0.083 (0.034)
HDL L C (mmol·L^{-1})	0.331 (0.154)	0.348 (0.157)
HDL M C (mmol·L^{-1})	0.527 (0.083)	0.530 (0.080)
HDL S C (mmol·L^{-1})	0.378 (0.037)	0.373 (0.039)
HDL VS C (mmol·L^{-1})	0.148 (0.012)	0.147 (0.012)
Total C (mmol·L^{-1})	4.358 (0.695)	4.400 (0.663)
Non-HDL C (mmol·L^{-1})	2.895 (0.686)	2.916 (0.663)
CM TG (mmol·L^{-1})	0.025 (0.042)	0.024 (0.034)
VLDL TG (mmol·L^{-1})	0.432 (0.312)	0.413 (0.280)
VLDL L1 TG (mmol·L^{-1})	0.038 (0.051)	0.036 (0.042)

VLDL L2 TG (mmol·L^{-1})	0.095 (0.100)	0.090 (0.086)
VLDL L3 TG (mmol·L^{-1})	0.155 (0.112)	0.147 (0.102)
VLDL M TG (mmol·L^{-1})	0.096 (0.049)	0.093 (0.048)
VLDL S TG (mmol·L^{-1})	0.046 (0.014)	0.046 (0.013)
LDL TG (mmol·L^{-1})	0.191 (0.034)	0.191 (0.032)
LDL L TG (mmol·L^{-1})	0.076 (0.017)	0.076 (0.016)
LDL M TG (mmol·L^{-1})	0.075 (0.013)	0.076 (0.013)
LDL S TG (mmol·L^{-1})	0.029 (0.006)	0.029 (0.005)
LDL VS TG (mmol·L^{-1})	0.011 (0.003)	0.011 (0.003)
HDL TG (mmol·L^{-1})	0.103 (0.037)	0.104 (0.033)
HDL VL TG (mmol·L^{-1})	0.006 (0.003)	0.006 (0.002)
HDL L TG (mmol·L^{-1})	0.026 (0.010)	0.026 (0.010)
HDL M TG (mmol·L^{-1})	0.038 (0.015)	0.038 (0.014)
HDL S TG (mmol·L^{-1})	0.022 (0.010)	0.022 (0.009)
HDL VS TG (mmol·L^{-1})	0.011 (0.003)	0.010 (0.003)
Total TG (mmol·L^{-1})	0.746 (0.402)	0.729 (0.351)
VLDL size (nm)	42.549 (3.034)	42.280 (2.602)
LDL size (nm)	25.767 (0.136)	25.784 (0.135)
HDL size (nm)	10.871 (0.211)	10.896 (0.216)

Abbreviations: CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

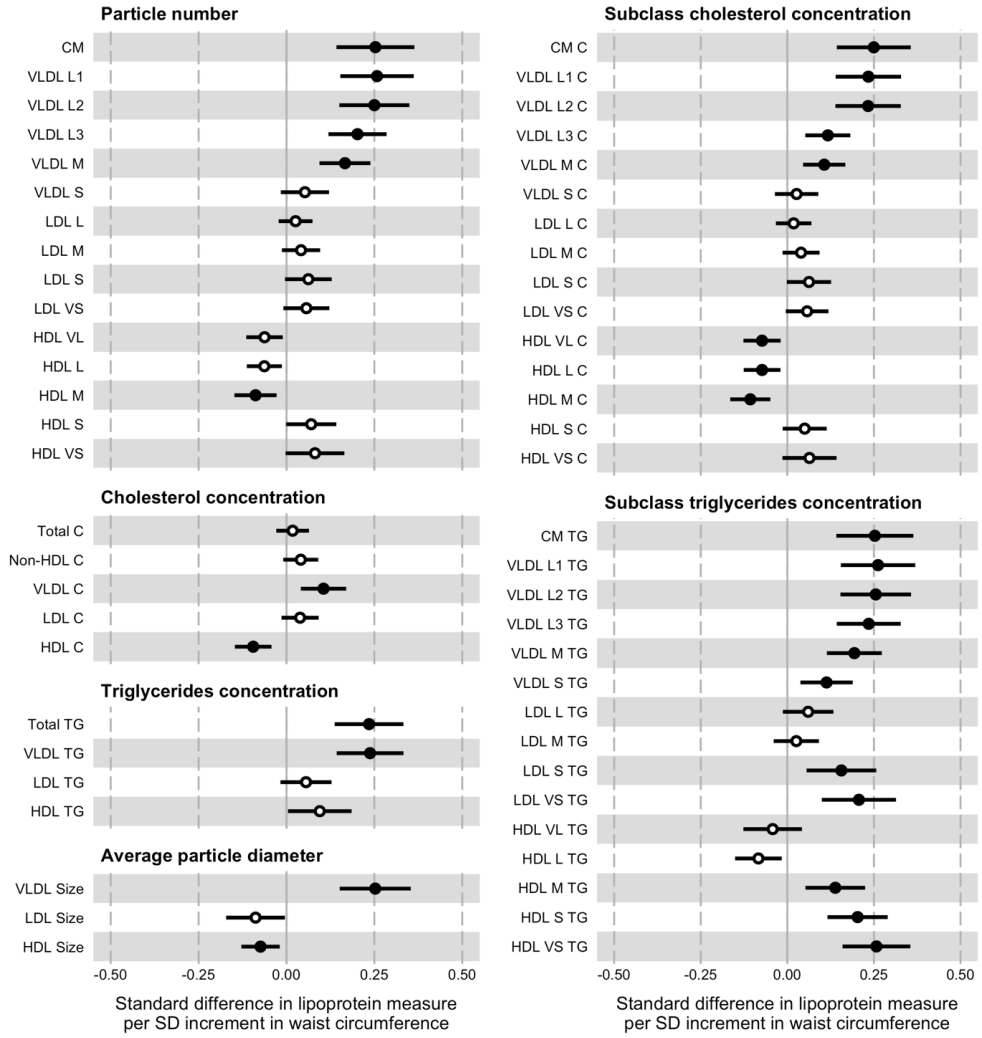
Figure 2. Cross-sectional associations of waist circumference with 57 lipoprotein measures



The associations were adjusted for age, parents' 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 waist circumference. Filled circles are p <0.01. Error bars are 95% CIs.

Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 3. Prospective associations of waist circumference with 57 lipoprotein measures



The associations were adjusted for age, parents' education, sex, sexual maturity, and baseline lipoprotein measure. 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 waist circumference. Filled circles are p <0.01. Error bars are 95% CIs.

Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 2. Cross-sectional associations between aerobic fitness and serum lipoprotein measures (n = 858)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	–0.0725	–0.1033	–0.0418	1.56E–05
VLDL L1 PN (nmol·L^{-1})	–0.2610	–0.3640	–0.1581	4.50E–06
VLDL L2 PN (nmol·L^{-1})	–0.9500	–1.3385	–0.5615	8.60E–06
VLDL L3 PN (nmol·L^{-1})	–2.5867	–3.4177	–1.7557	6.27E–08
VLDL M PN (nmol·L^{-1})	–2.7617	–3.7546	–1.7689	7.46E–07
VLDL S PN (nmol·L^{-1})	–1.2806	–2.3419	–0.2193	1.89E–02
LDL L PN (nmol·L^{-1})	–5.2875	–9.7038	–0.8712	1.98E–02
LDL M PN (nmol·L^{-1})	–17.2985	–26.3931	–8.2039	3.47E–04
LDL S PN (nmol·L^{-1})	–10.3361	–14.4806	–6.1916	6.08E–06
LDL VS PN (nmol·1^{-1})	–4.6706	–6.6237	–2.7174	1.26E–05
HDL VL PN (nmol·L^{-1})	17.3215	11.1888	23.4543	5.44E–07
HDL L PN (nmol·L^{-1})	163.2154	116.6032	209.8275	3.28E–09
HDL M PN (nmol·L^{-1})	88.5990	58.5261	118.6719	2.19E–07
HDL S PN (nmol·L^{-1})	–82.9860	–128.6851	–37.2869	6.00E–04
HDL VS PN (nmol·L^{-1})	–39.3204	–60.7217	–17.9191	5.25E–04
CM C (mmol·L^{-1})	–0.0028	–0.0039	–0.0016	1.74E–05
VLDL C (mmol·L^{-1})	–0.0598	–0.0791	–0.0405	6.64E–08
VLDL L1 C (mmol·L^{-1})	–0.0042	–0.0056	–0.0028	9.24E–08
VLDL L2 C (mmol·L^{-1})	–0.0090	–0.0124	–0.0057	1.41E–06
VLDL L3 C (mmol·L^{-1})	–0.0218	–0.0283	–0.0152	1.30E–08
VLDL M C (mmol·L^{-1})	–0.0169	–0.0223	–0.0116	3.97E–08
VLDL S C (mmol·L^{-1})	–0.0050	–0.0101	0.0001	5.39E–02
LDL C (mmol·L^{-1})	–0.0806	–0.1248	–0.0364	5.70E–04
LDL L C (mmol·L^{-1})	–0.0133	–0.0284	0.0018	8.36E–02
LDL M C (mmol·L^{-1})	–0.0350	–0.0552	–0.0149	9.86E–04
LDL S C (mmol·L^{-1})	–0.0189	–0.0267	–0.0111	9.80E–06
LDL VS C (mmol·L^{-1})	–0.0066	–0.0095	–0.0037	3.00E–05
HDL C (mmol·L^{-1})	0.0518	0.0371	0.0665	2.71E–09
HDL VL C (mmol·L^{-1})	0.0066	0.0044	0.0088	1.46E–07
HDL L C (mmol·L^{-1})	0.0357	0.0255	0.0459	3.12E–09
HDL M C (mmol·L^{-1})	0.0141	0.0098	0.0185	1.86E–08
HDL S C (mmol·L^{-1})	–0.0037	–0.0067	–0.0006	1.97E–02
HDL VS C (mmol·L^{-1})	–0.0013	–0.0023	–0.0002	1.81E–02
Total C (mmol·L^{-1})	–0.0899	–0.1471	–0.0328	2.60E–03
Non-HDL C (mmol·L^{-1})	–0.1418	–0.2004	–0.0832	1.03E–05
CM TG (mmol·L^{-1})	–0.0081	–0.0115	–0.0047	1.43E–05
VLDL TG (mmol·L^{-1})	–0.0683	–0.0947	–0.0419	3.11E–06
VLDL L1 TG (mmol·L^{-1})	–0.0106	–0.0150	–0.0062	1.02E–05
VLDL L2 TG (mmol·L^{-1})	–0.0212	–0.0299	–0.0124	1.03E–05

VLDL L3 TG (mmol·L^{-1})	–0.0249	–0.0344	–0.0154	2.51E–06
VLDL M TG (mmol·L^{-1})	–0.0096	–0.0135	–0.0056	9.46E–06
VLDL S TG (mmol·L^{-1})	–0.0021	–0.0032	–0.0009	7.31E–04
LDL TG (mmol·L^{-1})	–0.0059	–0.0089	–0.0029	2.48E–04
LDL L TG (mmol·L^{-1})	–0.0023	–0.0039	–0.0007	5.68E–03
LDL M TG (mmol·L^{-1})	–0.0018	–0.0030	–0.0006	3.08E–03
LDL S TG (mmol·L^{-1})	–0.0010	–0.0014	–0.0006	2.57E–05
LDL VS TG (mmol·L^{-1})	–0.0006	–0.0009	–0.0004	6.92E–06
HDL TG (mmol·L^{-1})	–0.0043	–0.0071	–0.0014	4.21E–03
HDL VL TG (mmol·L^{-1})	0.0000	–0.0002	0.0002	8.92E–01
HDL L TG (mmol·L^{-1})	0.0011	0.0004	0.0018	2.44E–03
HDL M TG (mmol·L^{-1})	–0.0022	–0.0033	–0.0010	3.42E–04
HDL S TG (mmol·L^{-1})	–0.0021	–0.0028	–0.0013	9.45E–07
HDL VS TG (mmol·L^{-1})	–0.0007	–0.0009	–0.0005	8.93E–08
Total TG (mmol·L^{-1})	–0.0854	–0.1188	–0.0521	3.70E–06
VLDL size (nm)	–0.6217	–0.8875	–0.3558	1.83E–05
LDL size (nm)	0.0160	0.0043	0.0276	8.02E–03
HDL size (nm)	0.0511	0.0363	0.0659	5.10E–09

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (103 m).
Adjusted for baseline values of age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 3. Cross-sectional associations between aerobic fitness and serum lipoprotein measures adjusted for waist circumference (n = 858)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	–0.0383	–0.0704	–0.0061	2.05E–02
VLDL L1 PN (nmol·L^{-1})	–0.1383	–0.2468	–0.0298	1.34E–02
VLDL L2 PN (nmol·L^{-1})	–0.5032	–0.9171	–0.0892	1.81E–02
VLDL L3 PN (nmol·L^{-1})	–1.3781	–2.2431	–0.5131	2.32E–03
VLDL M PN (nmol·L^{-1})	–1.5510	–2.5701	–0.5318	3.51E–03
VLDL S PN (nmol·L^{-1})	–0.9652	–2.0340	0.1037	7.58E–02
LDL L PN (nmol·L^{-1})	–3.5544	–7.7373	0.6286	9.43E–02
LDL M PN (nmol·L^{-1})	–9.3085	–17.5958	–1.0212	2.84E–02
LDL S PN (nmol·L^{-1})	–5.0986	–8.9389	–1.2584	1.02E–02
LDL VS PN (nmol·1^{-1})	–2.4665	–4.2910	–0.6421	8.95E–03
HDL VL PN (nmol·L^{-1})	5.3159	–1.5835	12.2152	1.28E–01
HDL L PN (nmol·L^{-1})	55.7928	5.0649	106.5206	3.17E–02
HDL M PN (nmol·L^{-1})	26.0505	–11.2178	63.3188	1.67E–01
HDL S PN (nmol·L^{-1})	–42.5062	–90.7219	5.7095	8.28E–02
HDL VS PN (nmol·L^{-1})	–18.9440	–41.5894	3.7013	9.93E–02
CM C (mmol·L^{-1})	–0.0015	–0.0027	–0.0002	1.99E–02
VLDL C (mmol·L^{-1})	–0.0337	–0.0518	–0.0156	4.52E–04
VLDL L1 C (mmol·L^{-1})	–0.0022	–0.0036	–0.0008	3.16E–03
VLDL L2 C (mmol·L^{-1})	–0.0049	–0.0084	–0.0013	8.24E–03
VLDL L3 C (mmol·L^{-1})	–0.0118	–0.0183	–0.0053	5.60E–04
VLDL M C (mmol·L^{-1})	–0.0089	–0.0139	–0.0038	8.56E–04
VLDL S C (mmol·L^{-1})	–0.0044	–0.0095	0.0007	9.21E–02
LDL C (mmol·L^{-1})	–0.0465	–0.0870	–0.0060	2.53E–02
LDL L C (mmol·L^{-1})	–0.0111	–0.0255	0.0033	1.29E–01
LDL M C (mmol·L^{-1})	–0.0199	–0.0386	–0.0012	3.71E–02
LDL S C (mmol·L^{-1})	–0.0096	–0.0168	–0.0023	1.04E–02
LDL VS C (mmol·L^{-1})	–0.0035	–0.0062	–0.0008	1.25E–02
HDL C (mmol·L^{-1})	0.0170	–0.0001	0.0341	5.15E–02
HDL VL C (mmol·L^{-1})	0.0021	–0.0004	0.0046	9.14E–02
HDL L C (mmol·L^{-1})	0.0127	0.0017	0.0238	2.47E–02
HDL M C (mmol·L^{-1})	0.0048	–0.0005	0.0100	7.50E–02
HDL S C (mmol·L^{-1})	–0.0019	–0.0051	0.0014	2.60E–01
HDL VS C (mmol·L^{-1})	–0.0006	–0.0017	0.0005	2.67E–01
Total C (mmol·L^{-1})	–0.0638	–0.1174	–0.0101	2.08E–02
Non-HDL C (mmol·L^{-1})	–0.0807	–0.1338	–0.0277	3.49E–03
CM TG (mmol·L^{-1})	–0.0043	–0.0078	–0.0007	2.03E–02
VLDL TG (mmol·L^{-1})	–0.0365	–0.0644	–0.0085	1.14E–02
VLDL L1 TG (mmol·L^{-1})	–0.0056	–0.0102	–0.0010	1.89E–02
VLDL L2 TG (mmol·L^{-1})	–0.0112	–0.0205	–0.0018	2.00E–02

VLDL L3 TG (mmol·L^{-1})	–0.0132	–0.0233	–0.0031	1.16E–02
VLDL M TG (mmol·L^{-1})	–0.0053	–0.0094	–0.0011	1.39E–02
VLDL S TG (mmol·L^{-1})	–0.0012	–0.0024	–0.0001	4.07E–02
LDL TG (mmol·L^{-1})	–0.0030	–0.0058	–0.0002	3.81E–02
LDL L TG (mmol·L^{-1})	–0.0014	–0.0030	0.0003	1.02E–01
LDL M TG (mmol·L^{-1})	–0.0009	–0.0020	0.0003	1.35E–01
LDL S TG (mmol·L^{-1})	–0.0006	–0.0010	–0.0001	1.85E–02
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0001	1.02E–02
HDL TG (mmol·L^{-1})	–0.0031	–0.0060	–0.0001	4.38E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	3.23E–01
HDL L TG (mmol·L^{-1})	0.0001	–0.0006	0.0009	7.27E–01
HDL M TG (mmol·L^{-1})	–0.0013	–0.0025	–0.0001	2.86E–02
HDL S TG (mmol·L^{-1})	–0.0011	–0.0019	–0.0003	7.23E–03
HDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0001	4.34E–03
Total TG (mmol·L^{-1})	–0.0465	–0.0816	–0.0115	1.02E–02
VLDL size (nm)	–0.3142	–0.6008	–0.0275	3.23E–02
LDL size (nm)	0.0031	–0.0103	0.0166	6.41E–01
HDL size (nm)	0.0193	0.0038	0.0348	1.54E–02

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (103 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 4. Cross-sectional associations between aerobic fitness and serum lipoprotein measures adjusted for body mass index (n = 858)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	–0.0367	–0.0681	–0.0053	2.28E–02
VLDL L1 PN (nmol·L^{-1})	–0.1327	–0.2374	–0.0279	1.40E–02
VLDL L2 PN (nmol·L^{-1})	–0.4806	–0.8800	–0.0812	1.92E–02
VLDL L3 PN (nmol·L^{-1})	–1.3057	–2.1195	–0.4920	2.17E–03
VLDL M PN (nmol·L^{-1})	–1.4268	–2.3782	–0.4754	3.98E–03
VLDL S PN (nmol·L^{-1})	–0.9163	–1.9566	0.1240	8.31E–02
LDL L PN (nmol·L^{-1})	–3.3372	–7.4910	0.8165	1.13E–01
LDL M PN (nmol·L^{-1})	–8.7874	–17.1033	–0.4715	3.87E–02
LDL S PN (nmol·L^{-1})	–4.8202	–8.6283	–1.0121	1.40E–02
LDL VS PN (nmol·1^{-1})	–2.2962	–4.0896	–0.5028	1.30E–02
HDL VL PN (nmol·L^{-1})	5.8949	–0.9907	12.7804	9.19E–02
HDL L PN (nmol·L^{-1})	58.5471	7.8283	109.2659	2.45E–02
HDL M PN (nmol·L^{-1})	24.2865	–12.3930	60.9660	1.90E–01
HDL S PN (nmol·L^{-1})	–48.0521	–95.6495	–0.4547	4.79E–02
HDL VS PN (nmol·L^{-1})	–20.7908	–43.4860	1.9043	7.18E–02
CM C (mmol·L^{-1})	–0.0014	–0.0026	–0.0002	2.32E–02
VLDL C (mmol·L^{-1})	–0.0310	–0.0479	–0.0141	5.39E–04
VLDL L1 C (mmol·L^{-1})	–0.0021	–0.0034	–0.0007	3.25E–03
VLDL L2 C (mmol·L^{-1})	–0.0046	–0.0079	–0.0012	9.22E–03
VLDL L3 C (mmol·L^{-1})	–0.0110	–0.0170	–0.0049	6.07E–04
VLDL M C (mmol·L^{-1})	–0.0078	–0.0126	–0.0030	1.85E–03
VLDL S C (mmol·L^{-1})	–0.0041	–0.0091	0.0008	1.03E–01
LDL C (mmol·L^{-1})	–0.0435	–0.0839	–0.0032	3.50E–02
LDL L C (mmol·L^{-1})	–0.0104	–0.0250	0.0041	1.55E–01
LDL M C (mmol·L^{-1})	–0.0188	–0.0377	0.0001	5.15E–02
LDL S C (mmol·L^{-1})	–0.0090	–0.0161	–0.0018	1.46E–02
LDL VS C (mmol·L^{-1})	–0.0032	–0.0059	–0.0006	1.80E–02
HDL C (mmol·L^{-1})	0.0171	0.0001	0.0341	4.82E–02
HDL VL C (mmol·L^{-1})	0.0023	–0.0002	0.0048	6.58E–02
HDL L C (mmol·L^{-1})	0.0133	0.0024	0.0243	1.80E–02
HDL M C (mmol·L^{-1})	0.0046	–0.0006	0.0097	8.06E–02
HDL S C (mmol·L^{-1})	–0.0023	–0.0055	0.0010	1.69E–01
HDL VS C (mmol·L^{-1})	–0.0007	–0.0018	0.0004	2.17E–01
Total C (mmol·L^{-1})	–0.0581	–0.1108	–0.0054	3.13E–02
Non-HDL C (mmol·L^{-1})	–0.0752	–0.1267	–0.0237	4.95E–03
CM TG (mmol·L^{-1})	–0.0041	–0.0076	–0.0006	2.30E–02
VLDL TG (mmol·L^{-1})	–0.0352	–0.0620	–0.0084	1.09E–02
VLDL L1 TG (mmol·L^{-1})	–0.0053	–0.0098	–0.0009	2.05E–02
VLDL L2 TG (mmol·L^{-1})	–0.0107	–0.0197	–0.0017	2.05E–02

VLDL L3 TG (mmol·L^{-1})	–0.0127	–0.0224	–0.0030	1.09E–02
VLDL M TG (mmol·L^{-1})	–0.0051	–0.0090	–0.0011	1.25E–02
VLDL S TG (mmol·L^{-1})	–0.0012	–0.0023	–0.0001	3.86E–02
LDL TG (mmol·L^{-1})	–0.0028	–0.0056	–0.0001	4.50E–02
LDL L TG (mmol·L^{-1})	–0.0013	–0.0028	0.0003	1.09E–01
LDL M TG (mmol·L^{-1})	–0.0008	–0.0019	0.0003	1.57E–01
LDL S TG (mmol·L^{-1})	–0.0005	–0.0010	0.0000	3.06E–02
LDL VS TG (mmol·L^{-1})	–0.0003	–0.0006	–0.0001	1.41E–02
HDL TG (mmol·L^{-1})	–0.0029	–0.0057	0.0000	4.96E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	4.34E–01
HDL L TG (mmol·L^{-1})	0.0002	–0.0005	0.0009	6.11E–01
HDL M TG (mmol·L^{-1})	–0.0013	–0.0024	–0.0001	2.82E–02
HDL S TG (mmol·L^{-1})	–0.0011	–0.0018	–0.0003	6.04E–03
HDL VS TG (mmol·L^{-1})	–0.0003	–0.0006	–0.0001	4.13E–03
Total TG (mmol·L^{-1})	–0.0445	–0.0781	–0.0109	1.04E–02
VLDL size (nm)	–0.3045	–0.5822	–0.0267	3.22E–02
LDL size (nm)	0.0027	–0.0103	0.0157	6.76E–01
HDL size (nm)	0.0202	0.0050	0.0354	1.01E–02

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (103 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and body mass index. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 5. Cross-sectional associations between waist circumference and serum lipoprotein measures (n = 894)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	0.1008	0.0664	0.1352	2.46E–07
VLDL L1 PN (nmol·L^{-1})	0.3559	0.2539	0.4579	3.57E–09
VLDL L2 PN (nmol·L^{-1})	1.2838	0.9226	1.6451	2.20E–09
VLDL L3 PN (nmol·L^{-1})	3.4820	2.7236	4.2404	8.68E–13
VLDL M PN (nmol·L^{-1})	3.5739	2.5615	4.5862	2.63E–09
VLDL S PN (nmol·L^{-1})	1.3280	0.5074	2.1485	2.00E–03
LDL L PN (nmol·L^{-1})	5.9392	2.5702	9.3083	8.35E–04
LDL M PN (nmol·L^{-1})	22.9680	14.9578	30.9783	3.95E–07
LDL S PN (nmol·L^{-1})	14.5329	10.6892	18.3767	3.89E–10
LDL VS PN (nmol·1^{-1})	6.2793	4.4282	8.1305	7.54E–09
HDL VL PN (nmol·L^{-1})	–30.9149	–36.5335	–25.2962	1.19E–15
HDL L PN (nmol·L^{-1})	–278.3540	–325.1463	–231.5618	5.46E–17
HDL M PN (nmol·L^{-1})	–153.0544	–192.9028	–113.2061	2.47E–10
HDL S PN (nmol·L^{-1})	114.1365	76.9297	151.3434	8.82E–08
HDL VS PN (nmol·L^{-1})	53.9795	35.9350	72.0240	1.56E–07
CM C (mmol·L^{-1})	0.0037	0.0025	0.0049	8.33E–08
VLDL C (mmol·L^{-1})	0.0781	0.0599	0.0962	7.44E–12
VLDL L1 C (mmol·L^{-1})	0.0057	0.0044	0.0071	1.83E–11
VLDL L2 C (mmol·L^{-1})	0.0121	0.0088	0.0154	1.34E–09
VLDL L3 C (mmol·L^{-1})	0.0288	0.0233	0.0343	7.71E–15
VLDL M C (mmol·L^{-1})	0.0232	0.0176	0.0288	2.25E–11
VLDL S C (mmol·L^{-1})	0.0041	0.0002	0.0080	3.98E–02
LDL C (mmol·L^{-1})	0.1017	0.0630	0.1404	2.32E–06
LDL L C (mmol·L^{-1})	0.0113	–0.0011	0.0236	7.25E–02
LDL M C (mmol·L^{-1})	0.0445	0.0269	0.0621	4.85E–06
LDL S C (mmol·L^{-1})	0.0261	0.0188	0.0334	1.83E–09
LDL VS C (mmol·L^{-1})	0.0088	0.0061	0.0116	2.81E–08
HDL C (mmol·L^{-1})	–0.0903	–0.1064	–0.0742	5.55E–16
HDL VL C (mmol·L^{-1})	–0.0116	–0.0136	–0.0095	7.89E–16
HDL L C (mmol·L^{-1})	–0.0604	–0.0703	–0.0504	2.45E–17
HDL M C (mmol·L^{-1})	–0.0235	–0.0288	–0.0182	2.84E–12
HDL S C (mmol·L^{-1})	0.0050	0.0024	0.0076	3.55E–04
HDL VS C (mmol·L^{-1})	0.0016	0.0006	0.0027	1.99E–03
Total C (mmol·L^{-1})	0.0921	0.0430	0.1411	4.11E–04
Non-HDL C (mmol·L^{-1})	0.1823	0.1352	0.2295	2.00E–10
CM TG (mmol·L^{-1})	0.0114	0.0074	0.0153	3.24E–07
VLDL TG (mmol·L^{-1})	0.0922	0.0675	0.1170	6.16E–10
VLDL L1 TG (mmol·L^{-1})	0.0145	0.0101	0.0189	1.68E–08
VLDL L2 TG (mmol·L^{-1})	0.0288	0.0207	0.0368	2.06E–09

VLDL L3 TG (mmol·L^{-1})	0.0337	0.0251	0.0422	1.27E–10
VLDL M TG (mmol·L^{-1})	0.0126	0.0089	0.0163	7.00E–09
VLDL S TG (mmol·L^{-1})	0.0026	0.0016	0.0037	5.23E–06
LDL TG (mmol·L^{-1})	0.0083	0.0056	0.0110	1.15E–07
LDL L TG (mmol·L^{-1})	0.0029	0.0017	0.0041	1.14E–05
LDL M TG (mmol·L^{-1})	0.0027	0.0016	0.0037	3.81E–06
LDL S TG (mmol·L^{-1})	0.0013	0.0008	0.0019	3.93E–06
LDL VS TG (mmol·L^{-1})	0.0009	0.0006	0.0012	4.36E–07
HDL TG (mmol·L^{-1})	0.0046	0.0011	0.0081	1.02E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0004	0.0001	2.45E–01
HDL L TG (mmol·L^{-1})	–0.0022	–0.0031	–0.0014	2.05E–06
HDL M TG (mmol·L^{-1})	0.0027	0.0013	0.0041	3.45E–04
HDL S TG (mmol·L^{-1})	0.0029	0.0020	0.0038	2.65E–08
HDL VS TG (mmol·L^{-1})	0.0010	0.0007	0.0012	6.06E–11
Total TG (mmol·L^{-1})	0.1143	0.0808	0.1479	6.45E–09
VLDL size (nm)	0.8731	0.6303	1.1159	1.60E–09
LDL size (nm)	–0.0299	–0.0408	–0.0189	1.13E–06
HDL size (nm)	–0.0831	–0.0972	–0.0690	8.22E–17

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of waist circumference (7.5 cm).
Adjusted for baseline values of age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 6. Cross-sectional associations between waist circumference and aerobic fitness (n = 858)

Measure	Coefficient	Lower CI	Upper CI	p value
Andersen test (m)	–40.1	–45.4	–34.8	2.23E–21

Regression coefficients are in metres run per standard deviation of waist circumference (7.4 cm).
Adjusted for baseline values of age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a threshold of 0.05.
In notation of p value 1.23E–02 stands for '1.23 times 10 to the power of –02' or 0.0123.
Abbreviations: CI = confidence interval.

Table 7. Prospective associations between baseline aerobic fitness and follow-up serum lipoprotein measures (n = 782)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	–0.0498	–0.0774	–0.0222	6.53E–04
VLDL L1 PN (nmol·L^{-1})	–0.1726	–0.2620	–0.0832	2.90E–04
VLDL L2 PN (nmol·L^{-1})	–0.6587	–0.9860	–0.3314	1.69E–04
VLDL L3 PN (nmol·L^{-1})	–1.3949	–2.0874	–0.7025	1.67E–04
VLDL M PN (nmol·L^{-1})	–1.6103	–2.3781	–0.8425	9.62E–05
VLDL S PN (nmol·L^{-1})	–0.4554	–1.2054	0.2945	2.29E–01
LDL L PN (nmol·L^{-1})	–0.8675	–3.3150	1.5800	4.81E–01
LDL M PN (nmol·L^{-1})	–2.8380	–7.2707	1.5947	2.05E–01
LDL S PN (nmol·L^{-1})	–2.1551	–4.3897	0.0795	5.84E–02
LDL VS PN (nmol·1^{-1})	–1.1115	–2.1587	–0.0643	3.79E–02
HDL VL PN (nmol·L^{-1})	0.9199	–3.4390	5.2787	6.74E–01
HDL L PN (nmol·L^{-1})	6.4885	–28.2483	41.2253	7.10E–01
HDL M PN (nmol·L^{-1})	18.5158	–13.4459	50.4775	2.51E–01
HDL S PN (nmol·L^{-1})	–24.6034	–63.7032	14.4964	2.13E–01
HDL VS PN (nmol·L^{-1})	–16.8579	–31.9547	–1.7610	2.93E–02
CM C (mmol·L^{-1})	–0.0019	–0.0029	–0.0009	4.11E–04
VLDL C (mmol·L^{-1})	–0.0194	–0.0326	–0.0061	4.86E–03
VLDL L1 C (mmol·L^{-1})	–0.0024	–0.0036	–0.0012	2.33E–04
VLDL L2 C (mmol·L^{-1})	–0.0057	–0.0085	–0.0029	1.71E–04
VLDL L3 C (mmol·L^{-1})	–0.0080	–0.0123	–0.0036	5.57E–04
VLDL M C (mmol·L^{-1})	–0.0064	–0.0105	–0.0022	3.07E–03
VLDL S C (mmol·L^{-1})	–0.0015	–0.0051	0.0021	4.11E–01
LDL C (mmol·L^{-1})	–0.0157	–0.0372	0.0058	1.49E–01
LDL L C (mmol·L^{-1})	–0.0030	–0.0121	0.0061	5.10E–01
LDL M C (mmol·L^{-1})	–0.0066	–0.0167	0.0034	1.92E–01
LDL S C (mmol·L^{-1})	–0.0042	–0.0083	–0.0001	4.37E–02
LDL VS C (mmol·L^{-1})	–0.0017	–0.0032	–0.0001	3.39E–02
HDL C (mmol·L^{-1})	0.0046	–0.0074	0.0165	4.46E–01
HDL VL C (mmol·L^{-1})	0.0004	–0.0012	0.0019	6.59E–01
HDL L C (mmol·L^{-1})	0.0016	–0.0059	0.0091	6.73E–01
HDL M C (mmol·L^{-1})	0.0023	–0.0019	0.0064	2.81E–01
HDL S C (mmol·L^{-1})	–0.0012	–0.0037	0.0013	3.31E–01
HDL VS C (mmol·L^{-1})	–0.0007	–0.0014	0.0000	4.17E–02
Total C (mmol·L^{-1})	–0.0237	–0.0507	0.0033	8.43E–02
Non-HDL C (mmol·L^{-1})	–0.0231	–0.0475	0.0012	6.23E–02
CM TG (mmol·L^{-1})	–0.0056	–0.0087	–0.0025	6.47E–04
VLDL TG (mmol·L^{-1})	–0.0444	–0.0672	–0.0216	2.56E–04
VLDL L1 TG (mmol·L^{-1})	–0.0072	–0.0110	–0.0034	3.63E–04
VLDL L2 TG (mmol·L^{-1})	–0.0145	–0.0219	–0.0070	2.61E–04

VLDL L3 TG (mmol·L^{-1})	–0.0161	–0.0242	–0.0080	1.99E–04
VLDL M TG (mmol·L^{-1})	–0.0067	–0.0101	–0.0033	2.05E–04
VLDL S TG (mmol·L^{-1})	–0.0011	–0.0021	–0.0002	1.90E–02
LDL TG (mmol·L^{-1})	–0.0013	–0.0033	0.0007	2.10E–01
LDL L TG (mmol·L^{-1})	–0.0008	–0.0020	0.0005	2.22E–01
LDL M TG (mmol·L^{-1})	–0.0002	–0.0011	0.0007	6.55E–01
LDL S TG (mmol·L^{-1})	–0.0006	–0.0010	–0.0002	3.28E–03
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0007	–0.0002	8.71E–04
HDL TG (mmol·L^{-1})	–0.0027	–0.0052	–0.0002	3.18E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0002	0.0001	4.83E–01
HDL L TG (mmol·L^{-1})	0.0001	–0.0005	0.0006	8.03E–01
HDL M TG (mmol·L^{-1})	–0.0013	–0.0023	–0.0003	1.37E–02
HDL S TG (mmol·L^{-1})	–0.0011	–0.0018	–0.0004	2.88E–03
HDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0002	2.05E–04
Total TG (mmol·L^{-1})	–0.0556	–0.0848	–0.0264	3.42E–04
VLDL size (nm)	–0.4369	–0.6642	–0.2096	3.06E–04
LDL size (nm)	0.0081	–0.0027	0.0190	1.40E–01
HDL size (nm)	0.0034	–0.0072	0.0141	5.20E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (102 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 8. Prospective associations between baseline aerobic fitness and follow-up serum lipoprotein measures adjusted for waist circumference (n = 782)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	–0.0224	–0.0484	0.0035	8.90E–02
VLDL L1 PN (nmol·L^{-1})	–0.0774	–0.1581	0.0032	5.94E–02
VLDL L2 PN (nmol·L^{-1})	–0.3084	–0.5973	–0.0196	3.68E–02
VLDL L3 PN (nmol·L^{-1})	–0.6748	–1.3028	–0.0469	3.57E–02
VLDL M PN (nmol·L^{-1})	–0.8176	–1.5097	–0.1255	2.14E–02
VLDL S PN (nmol·L^{-1})	–0.2286	–1.0488	0.5916	5.79E–01
LDL L PN (nmol·L^{-1})	–0.4463	–3.1513	2.2587	7.42E–01
LDL M PN (nmol·L^{-1})	–1.3098	–6.5109	3.8913	6.16E–01
LDL S PN (nmol·L^{-1})	–1.0091	–3.6703	1.6521	4.51E–01
LDL VS PN (nmol·1^{-1})	–0.6381	–1.8771	0.6008	3.07E–01
HDL VL PN (nmol·L^{-1})	–1.8259	–6.6052	2.9534	4.47E–01
HDL L PN (nmol·L^{-1})	–14.2781	–52.7322	24.1760	4.60E–01
HDL M PN (nmol·L^{-1})	–6.0963	–39.3374	27.1448	7.15E–01
HDL S PN (nmol·L^{-1})	–8.9497	–47.9038	30.0043	6.47E–01
HDL VS PN (nmol·L^{-1})	–8.5965	–24.6609	7.4680	2.88E–01
CM C (mmol·L^{-1})	–0.0008	–0.0018	0.0001	7.84E–02
VLDL C (mmol·L^{-1})	–0.0103	–0.0237	0.0032	1.31E–01
VLDL L1 C (mmol·L^{-1})	–0.0011	–0.0022	0.0000	4.61E–02
VLDL L2 C (mmol·L^{-1})	–0.0026	–0.0051	–0.0001	4.18E–02
VLDL L3 C (mmol·L^{-1})	–0.0042	–0.0086	0.0002	5.90E–02
VLDL M C (mmol·L^{-1})	–0.0037	–0.0076	0.0003	6.73E–02
VLDL S C (mmol·L^{-1})	–0.0010	–0.0051	0.0031	6.19E–01
LDL C (mmol·L^{-1})	–0.0091	–0.0339	0.0156	4.63E–01
LDL L C (mmol·L^{-1})	–0.0021	–0.0120	0.0078	6.79E–01
LDL M C (mmol·L^{-1})	–0.0034	–0.0150	0.0082	5.64E–01
LDL S C (mmol·L^{-1})	–0.0021	–0.0069	0.0026	3.75E–01
LDL VS C (mmol·L^{-1})	–0.0010	–0.0027	0.0008	2.65E–01
HDL C (mmol·L^{-1})	–0.0061	–0.0182	0.0061	3.20E–01
HDL VL C (mmol·L^{-1})	–0.0008	–0.0025	0.0009	3.49E–01
HDL L C (mmol·L^{-1})	–0.0034	–0.0114	0.0045	3.88E–01
HDL M C (mmol·L^{-1})	–0.0017	–0.0059	0.0025	4.13E–01
HDL S C (mmol·L^{-1})	–0.0005	–0.0029	0.0020	7.12E–01
HDL VS C (mmol·L^{-1})	–0.0004	–0.0011	0.0003	2.81E–01
Total C (mmol·L^{-1})	–0.0218	–0.0541	0.0105	1.81E–01
Non-HDL C (mmol·L^{-1})	–0.0132	–0.0433	0.0170	3.86E–01
CM TG (mmol·L^{-1})	–0.0025	–0.0054	0.0004	9.20E–02
VLDL TG (mmol·L^{-1})	–0.0202	–0.0404	0.0001	5.09E–02
VLDL L1 TG (mmol·L^{-1})	–0.0032	–0.0067	0.0003	6.88E–02
VLDL L2 TG (mmol·L^{-1})	–0.0065	–0.0132	0.0002	5.63E–02

VLDL L3 TG (mmol·L^{-1})	–0.0073	–0.0145	–0.0001	4.62E–02
VLDL M TG (mmol·L^{-1})	–0.0032	–0.0062	–0.0002	3.91E–02
VLDL S TG (mmol·L^{-1})	–0.0005	–0.0014	0.0004	2.43E–01
LDL TG (mmol·L^{-1})	–0.0006	–0.0027	0.0015	5.63E–01
LDL L TG (mmol·L^{-1})	–0.0004	–0.0016	0.0008	5.12E–01
LDL M TG (mmol·L^{-1})	–0.0001	–0.0011	0.0009	8.80E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0008	0.0001	1.04E–01
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0005	0.0000	5.95E–02
HDL TG (mmol·L^{-1})	–0.0016	–0.0040	0.0008	1.81E–01
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	1.75E–01
HDL L TG (mmol·L^{-1})	–0.0003	–0.0009	0.0003	3.27E–01
HDL M TG (mmol·L^{-1})	–0.0006	–0.0016	0.0004	2.10E–01
HDL S TG (mmol·L^{-1})	–0.0004	–0.0010	0.0002	2.10E–01
HDL VS TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	4.65E–02
Total TG (mmol·L^{-1})	–0.0259	–0.0521	0.0003	5.27E–02
VLDL size (nm)	–0.1971	–0.4049	0.0107	6.26E–02
LDL size (nm)	0.0031	–0.0091	0.0154	6.08E–01
HDL size (nm)	–0.0034	–0.0149	0.0081	5.59E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (102 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, waist circumference, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 9. Prospective associations between baseline aerobic fitness and follow-up serum lipoprotein measures adjusted for body mass index (n = 782)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	–0.0259	–0.0519	0.0001	5.08E–02
VLDL L1 PN (nmol·L^{-1})	–0.0880	–0.1681	–0.0079	3.19E–02
VLDL L2 PN (nmol·L^{-1})	–0.3472	–0.6359	–0.0586	1.93E–02
VLDL L3 PN (nmol·L^{-1})	–0.7284	–1.3559	–0.1008	2.37E–02
VLDL M PN (nmol·L^{-1})	–0.8504	–1.5494	–0.1514	1.80E–02
VLDL S PN (nmol·L^{-1})	–0.1645	–0.9728	0.6439	6.85E–01
LDL L PN (nmol·L^{-1})	–0.1604	–2.8711	2.5503	9.06E–01
LDL M PN (nmol·L^{-1})	–1.0024	–6.2174	4.2127	7.02E–01
LDL S PN (nmol·L^{-1})	–0.9969	–3.5854	1.5916	4.44E–01
LDL VS PN (nmol·1^{-1})	–0.6119	–1.8184	0.5946	3.14E–01
HDL VL PN (nmol·L^{-1})	–1.8116	–6.4671	2.8438	4.39E–01
HDL L PN (nmol·L^{-1})	–14.5990	–52.0804	22.8823	4.39E–01
HDL M PN (nmol·L^{-1})	–7.3814	–40.6342	25.8714	6.58E–01
HDL S PN (nmol·L^{-1})	–11.7312	–50.3986	26.9362	5.46E–01
HDL VS PN (nmol·L^{-1})	–10.0372	–24.9175	4.8431	1.82E–01
CM C (mmol·L^{-1})	–0.0009	–0.0019	0.0000	4.93E–02
VLDL C (mmol·L^{-1})	–0.0097	–0.0234	0.0040	1.60E–01
VLDL L1 C (mmol·L^{-1})	–0.0012	–0.0023	–0.0001	3.15E–02
VLDL L2 C (mmol·L^{-1})	–0.0028	–0.0054	–0.0003	2.80E–02
VLDL L3 C (mmol·L^{-1})	–0.0042	–0.0086	0.0002	6.16E–02
VLDL M C (mmol·L^{-1})	–0.0034	–0.0074	0.0006	9.28E–02
VLDL S C (mmol·L^{-1})	–0.0006	–0.0046	0.0035	7.83E–01
LDL C (mmol·L^{-1})	–0.0072	–0.0320	0.0177	5.66E–01
LDL L C (mmol·L^{-1})	–0.0007	–0.0107	0.0092	8.83E–01
LDL M C (mmol·L^{-1})	–0.0025	–0.0141	0.0091	6.65E–01
LDL S C (mmol·L^{-1})	–0.0020	–0.0067	0.0026	3.87E–01
LDL VS C (mmol·L^{-1})	–0.0009	–0.0026	0.0008	3.04E–01
HDL C (mmol·L^{-1})	–0.0058	–0.0175	0.0060	3.30E–01
HDL VL C (mmol·L^{-1})	–0.0007	–0.0024	0.0009	3.81E–01
HDL L C (mmol·L^{-1})	–0.0032	–0.0110	0.0046	4.15E–01
HDL M C (mmol·L^{-1})	–0.0018	–0.0060	0.0024	4.00E–01
HDL S C (mmol·L^{-1})	–0.0006	–0.0031	0.0018	6.07E–01
HDL VS C (mmol·L^{-1})	–0.0005	–0.0011	0.0002	1.84E–01
Total C (mmol·L^{-1})	–0.0189	–0.0508	0.0130	2.40E–01
Non-HDL C (mmol·L^{-1})	–0.0109	–0.0413	0.0195	4.77E–01
CM TG (mmol·L^{-1})	–0.0029	–0.0058	0.0001	5.57E–02
VLDL TG (mmol·L^{-1})	–0.0226	–0.0428	–0.0024	2.89E–02
VLDL L1 TG (mmol·L^{-1})	–0.0037	–0.0071	–0.0002	3.95E–02
VLDL L2 TG (mmol·L^{-1})	–0.0074	–0.0140	–0.0007	3.07E–02

VLDL L3 TG (mmol·L^{-1})	–0.0082	–0.0154	–0.0010	2.60E–02
VLDL M TG (mmol·L^{-1})	–0.0035	–0.0066	–0.0005	2.50E–02
VLDL S TG (mmol·L^{-1})	–0.0005	–0.0014	0.0004	2.44E–01
LDL TG (mmol·L^{-1})	–0.0006	–0.0027	0.0016	6.07E–01
LDL L TG (mmol·L^{-1})	–0.0003	–0.0015	0.0009	6.15E–01
LDL M TG (mmol·L^{-1})	0.0000	–0.0010	0.0010	9.57E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0008	0.0001	9.83E–02
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0005	0.0000	4.65E–02
HDL TG (mmol·L^{-1})	–0.0017	–0.0041	0.0007	1.64E–01
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	1.93E–01
HDL L TG (mmol·L^{-1})	–0.0003	–0.0009	0.0004	3.76E–01
HDL M TG (mmol·L^{-1})	–0.0007	–0.0016	0.0003	1.74E–01
HDL S TG (mmol·L^{-1})	–0.0004	–0.0011	0.0002	1.59E–01
HDL VS TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	2.67E–02
Total TG (mmol·L^{-1})	–0.0288	–0.0549	–0.0026	3.19E–02
VLDL size (nm)	–0.2285	–0.4371	–0.0200	3.23E–02
LDL size (nm)	0.0045	–0.0077	0.0167	4.60E–01
HDL size (nm)	–0.0030	–0.0144	0.0083	5.94E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of distance run in the Andersen test (102 m).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, body mass index, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 10. Prospective associations between baseline waist circumference and follow-up serum lipoprotein measures (n = 807)

Lipoprotein measure	Coefficient	Lower CI	Upper CI	p value
CM PN (nmol·L^{-1})	0.0783	0.0440	0.1126	2.73E–05
VLDL L1 PN (nmol·L^{-1})	0.2670	0.1587	0.3753	7.44E–06
VLDL L2 PN (nmol·L^{-1})	0.9721	0.5842	1.3599	5.55E–06
VLDL L3 PN (nmol·L^{-1})	2.0129	1.1881	2.8378	8.92E–06
VLDL M PN (nmol·L^{-1})	2.1247	1.1988	3.0506	2.49E–05
VLDL S PN (nmol·L^{-1})	0.5890	–0.1824	1.3604	1.32E–01
LDL L PN (nmol·L^{-1})	1.1370	–0.9853	3.2593	2.88E–01
LDL M PN (nmol·L^{-1})	4.0986	–1.3065	9.5038	1.34E–01
LDL S PN (nmol·L^{-1})	3.0830	–0.2160	6.3821	6.64E–02
LDL VS PN (nmol·1^{-1})	1.3421	–0.2155	2.8997	8.99E–02
HDL VL PN (nmol·L^{-1})	–5.9558	–10.9209	–0.9907	1.96E–02
HDL L PN (nmol·L^{-1})	–47.3548	–84.8384	–9.8711	1.42E–02
HDL M PN (nmol·L^{-1})	–51.6325	–86.8176	–16.4473	4.77E–03
HDL S PN (nmol·L^{-1})	39.6368	–0.6107	79.8842	5.35E–02
HDL VS PN (nmol·L^{-1})	20.6371	–0.6858	41.9599	5.76E–02
CM C (mmol·L^{-1})	0.0029	0.0016	0.0041	1.87E–05
VLDL C (mmol·L^{-1})	0.0254	0.0098	0.0410	1.87E–03
VLDL L1 C (mmol·L^{-1})	0.0036	0.0022	0.0051	6.80E–06
VLDL L2 C (mmol·L^{-1})	0.0085	0.0050	0.0119	7.24E–06
VLDL L3 C (mmol·L^{-1})	0.0102	0.0045	0.0159	6.68E–04
VLDL M C (mmol·L^{-1})	0.0077	0.0033	0.0121	9.45E–04
VLDL S C (mmol·L^{-1})	0.0015	–0.0020	0.0049	3.97E–01
LDL C (mmol·L^{-1})	0.0189	–0.0069	0.0447	1.48E–01
LDL L C (mmol·L^{-1})	0.0029	–0.0053	0.0112	4.80E–01
LDL M C (mmol·L^{-1})	0.0088	–0.0031	0.0206	1.43E–01
LDL S C (mmol·L^{-1})	0.0058	–0.0001	0.0117	5.50E–02
LDL VS C (mmol·L^{-1})	0.0020	–0.0002	0.0041	6.93E–02
HDL C (mmol·L^{-1})	–0.0241	–0.0374	–0.0109	5.91E–04
HDL VL C (mmol·L^{-1})	–0.0025	–0.0044	–0.0007	8.69E–03
HDL L C (mmol·L^{-1})	–0.0115	–0.0199	–0.0031	8.08E–03
HDL M C (mmol·L^{-1})	–0.0086	–0.0133	–0.0040	4.99E–04
HDL S C (mmol·L^{-1})	0.0020	–0.0005	0.0044	1.20E–01
HDL VS C (mmol·L^{-1})	0.0008	–0.0002	0.0017	1.07E–01
Total C (mmol·L^{-1})	0.0115	–0.0192	0.0422	4.55E–01
Non-HDL C (mmol·L^{-1})	0.0269	–0.0061	0.0598	1.08E–01
CM TG (mmol·L^{-1})	0.0088	0.0049	0.0127	2.95E–05
VLDL TG (mmol·L^{-1})	0.0668	0.0401	0.0935	5.84E–06
VLDL L1 TG (mmol·L^{-1})	0.0112	0.0066	0.0158	9.21E–06
VLDL L2 TG (mmol·L^{-1})	0.0220	0.0132	0.0308	5.66E–06

VLDL L3 TG (mmol·L^{-1})	0.0241	0.0146	0.0335	4.23E–06
VLDL M TG (mmol·L^{-1})	0.0092	0.0054	0.0130	9.14E–06
VLDL S TG (mmol·L^{-1})	0.0015	0.0005	0.0025	3.97E–03
LDL TG (mmol·L^{-1})	0.0018	–0.0006	0.0041	1.33E–01
LDL L TG (mmol·L^{-1})	0.0010	–0.0002	0.0021	1.05E–01
LDL M TG (mmol·L^{-1})	0.0003	–0.0005	0.0012	4.31E–01
LDL S TG (mmol·L^{-1})	0.0008	0.0003	0.0013	2.97E–03
LDL VS TG (mmol·L^{-1})	0.0006	0.0003	0.0009	2.92E–04
HDL TG (mmol·L^{-1})	0.0032	0.0001	0.0062	4.06E–02
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0003	0.0001	3.21E–01
HDL L TG (mmol·L^{-1})	–0.0009	–0.0015	–0.0002	1.61E–02
HDL M TG (mmol·L^{-1})	0.0019	0.0007	0.0031	2.12E–03
HDL S TG (mmol·L^{-1})	0.0018	0.0011	0.0026	1.91E–05
HDL VS TG (mmol·L^{-1})	0.0007	0.0004	0.0009	2.33E–06
Total TG (mmol·L^{-1})	0.0828	0.0483	0.1173	1.18E–05
VLDL size (nm)	0.6618	0.3968	0.9268	5.94E–06
LDL size (nm)	–0.0120	–0.0233	–0.0006	3.96E–02
HDL size (nm)	–0.0160	–0.0278	–0.0042	8.92E–03

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of waist circumference (7.5 cm).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and respective lipoprotein measure. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicrons; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 11. Prospective associations between baseline waist circumference and follow-up aerobic fitness (*n* = 737)

Measure	Coefficient	Lower CI	Upper CI	*p* value
Andersen test (m)	–16.7	–21.6	–11.8	6.68E–09

Regression coefficients are in metres run per standard deviation of waist circumference (7.4 cm).
Adjusted for baseline values of age, parents' education, sex, sexual maturity, and aerobic fitness. Cluster-robust standard errors were calculated, clustered on the school variable.
p values should be interpreted at a threshold of 0.05.
In notation of *p* value 1.23E–02 stands for '1.23 times 10 to the power of –02' or 0.0123.
Abbreviations: CI = confidence interval.

Paper III

Associations of lipoprotein profile and objectively measured physical activity and sedentary time in schoolchildren: a prospective cohort study.

Abstract

Background: Our understanding of the mechanisms through which physical activity might benefit lipoprotein metabolism is inadequate. Here we characterise the continuous associations between physical activity of different intensities, sedentary time, and a comprehensive lipoprotein profile.

Methods: Our cohort included 762 fifth grade (mean [SD] age = 10.0 [0.3] y) Norwegian schoolchildren (49.6% girls) measured on two separate occasions across one school year. We used targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to produce 57 lipoprotein measures from fasted blood serum samples, including subclass particle numbers, cholesterol and triglycerides concentrations. The children wore triaxial accelerometers for seven consecutive days to record time spent in light-, moderate-, and vigorous-intensity physical activity, and sedentary time. We used separate multivariable linear regression to analyse associations between the device-measured activity variables—modelled both prospectively (baseline value) and as a change score (follow-up minus baseline value)—and each lipoprotein measure at follow-up. All models were adjusted for the respective lipoprotein measure at baseline, accelerometer wear time, age, parents' education, sex, and sexual maturity. We additionally adjusted for waist circumference in separate models.

Results: Higher baseline levels of moderate- and vigorous-intensity physical activity were associated with a favourable lipoprotein profile at follow-up. The strongest associations were with the larger subclasses of triglyceride-rich lipoproteins and inverse. Sedentary time was associated with an unfavourable lipoprotein profile, the pattern and magnitude of associations being similar to, but in the opposite direction of, those in the moderate- and vigorous-intensity physical activity analyses. The associations with light-intensity physical activity were more modest, and those of the change models were weak. Additional adjustment for waist circumference as a covariate tended to have only a small attenuating effect on association magnitudes in all models.

Conclusion: We provide evidence of a prospective association between time spent active or sedentary and lipoprotein metabolism in schoolchildren. Change in activity levels across the school year is of limited influence in our young, healthy cohort.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally.[1] The associations between physical activity (PA), CVD incidence and mortality are well-established,[2] and raising levels of PA is considered a cornerstone of disease prevention for both individuals and populations.[3,4] In children and adolescents, higher levels of PA are associated with better composite scores of cardiometabolic health.[5,6] However, the relationship with individual cardiometabolic risk factors, such as blood lipids, is inconsistent and most data are from cross-sectional studies.[7] It is also unclear whether these associations are independent of adiposity.[8]

More detailed metabolic phenotyping—metabolomics—can improve our understanding of the mechanisms by which PA benefits metabolism by providing detailed information of the molecules and pathways involved.[9] A small number of studies have revealed associations of PA with a number of novel measures of lipid metabolism not observable with the standard lipid profile.[10,11] We have previously examined the theoretical effects of replacing time spent sedentary with moderate- to vigorous-intensity physical activity (MVPA) using isotemporal substitution, but were limited in our ability to examine temporality of the associations due to the cross-sectional design.[12]

In this study, we used targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to produce comprehensive lipoprotein profiles for our cohort of healthy schoolchildren, then examined the continuous associations with objectively measured PA of different intensities and sedentary time over one school year. We also explored potential confounding of associations by adiposity.

2. Methods

Additional information regarding blood sample handling and the ^{1}H NMR protocol are reported in the Supplementary Material.

2.1. Sample population

We drew our cohort from children who participated in the Active Smarter Kids (ASK) study; a cluster randomised controlled trial (RCT) in which the effect of a school-based PA intervention on academic performance was investigated (https://clinicaltrials.gov, #NCT02132494).[13] Of the 60 schools approached, 57 (1129 children) participated. The PA intervention was delivered over one academic year. Baseline testing took place in 2014. Changes in physical activity levels were of a similar degree for children who either received the intervention or did not. We therefore pooled all children for this analysis.

2.2. Ethics

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.[14]

2.3. Exposure variables

The children wore triaxial accelerometers (ActiGraph GT3X+, ActiGraph LLC, Pensacola, FL) positioned on their right hip for seven consecutive days, but not during sleep or water-based

activities. We considered a valid day ≥480 min of monitor wear time between 0600 and 0000. Non-wear time was defined as ≥20 min of zero counts.[15] The accelerometer data were processed using commercially available KineSoft software (version 3.3.80, KineSoft, Loughborough, United Kingdom) and 10-s epochs. We classified PA intensity and sedentary time using the Evenson cut points of count data: sedentary time (≤100 counts·min^{-1}), low-intensity physical activity (LPA; >100 and <2296 counts·min^{-1}), moderate-intensity physical activity (MPA; ≥2296 and <4012 counts·min^{-1}), and vigorous-intensity physical activity (VPA; ≥4012 counts·min^{-1}).[16,17]

2.4. Outcome variables

The children fasted overnight and a trained nurse or phlebotomist drew blood serum samples between 0800 and 1000. NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). We selected the lipoprotein NMR spectral regions as explanatory variables to partial least squares (PLS) modelling. The PLS model response variables were determined by high-performance liquid chromatography (HPLC).[18,19] These comprised: total serum cholesterol concentration; total serum triglycerides concentration; cholesterol concentration, and triglycerides concentration for 20 lipoprotein subclasses. We subsequently reduced the 20 lipoprotein subclasses to 15 as previously described,[20] and calculated non-HDL cholesterol concentration in addition, by subtracting the HDL cholesterol concentration from total cholesterol concentration. In total, 106 serum samples were randomly selected for both HPLC and NMR analysis. We used a Monte Carlo resampling approach to calculate individual PLS models with optimal prediction ability for the HPLC data.[21] Lipoprotein particle numbers for all samples were predicted from these models. Due to the elution of lipid-poor pre-β_1 high-density lipoproteins (HDLs),[22] the "spherical particle model" for calculating particle number cannot be applied to the HDL7 minor subclass.[19] Hence, the particle number of the HDL VS subclass in our study was calculated using HDL6 only. Though NMR cannot distinguish intact chylomicron particles from the largest very low-density lipoprotein (VLDL) particles, we have retained the classification nomenclature derived from the HPLC method. Particles in this class should be considered either chylomicrons or very large VLDLs.

2.5. Anthropometrics

We measured body weight to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany). Height—with shoes removed, facing forwards—to the nearest 0.1 cm using a stadiometer (Seca 217, SECA GmbH, Hamburg, Germany). We calculated body mass index (BMI) as weight divided by height squared (kg·m^{-2}). Using a measuring tape (Seca 201, SECA GmbH, Hamburg, Germany), we took two measurements of waist circumference—between the lowest palpable rib and iliac crest, the child having gently exhaled—to the nearest 0.1 cm. If the two measurements differed by more than 1.0 cm a third was taken; the mean of the two with the least difference was used for analysis. The proportions of overweight or obese girls and boys were calculated using the International Obesity Task Force's (IOTF) sex-specific BMI cut-offs, rounding down the children's ages at the time of testing to the nearest half-year.[23]

2.6. Sexual maturity

Assessments were conducted in a private room and children were accompanied by a researcher of the same sex to ensure their comfort. Each child assessed their sexual maturity against a standard set of colour images and accompanying text descriptions that corresponded to the Tanner staging method.[24] We recorded low frequencies of children in Tanner categories 3, 4, and 5 (n = 66, 5, 2, respectively of 1081 children with valid baseline data) and combined them into one category (≥3) for analysis.

2.7. Socioeconomic status

We quantified socioeconomic status (SES) as the highest level of educational attainment of a child's mother, father, or guardian, whichever was higher. Each parent or guardian completed a custom questionnaire for the ASK study, selecting their level of attainment from six categories. Of these six, we recorded low frequencies in the lower four categories (n = 4, 15, 193, 137, for categories 1–4, respectively of 1069 children with valid baseline data), so combined them into one category—*Upper secondary school*—for analysis.

2.8. Statistical approach

We examined the associations between the mean minutes per valid day of accelerometer data for each of the four activity variables (LPA, MPA, VPA, and sedentary time) measured at baseline and the 57 lipoprotein variables measured at follow-up using multivariable linear regression. We built separate models for each lipoprotein measure. Models were adjusted for the baseline value of the respective lipoprotein measure, mean daily accelerometer wear time, age, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated for each model to account for potential within-cluster correlation of the school variable and to obviate transforming skewed outcome measures. We further adjusted the models for waist circumference at baseline to investigate potential confounding by adiposity.

We also examined associations between change in the activity variables over the follow-up period. Instead of using baseline values of activity intensity, we used change scores (follow-up minus baseline). As per the prospective models, we modelled the follow-up values of individual lipoprotein measures as the outcome variables, adjusting for their respective baseline values. Accelerometer wear time was modelled as a change score, whereas baseline values were used for the other covariates. In separate analyses that included waist circumference, baseline values were used given the high correlations with follow-up values (Pearson's r = 0.93, p <0.001).

Prior to analysis, all PA and lipoprotein variables were converted to z-scores (mean = 0.0; standard deviation [SD] = 1.0), hence the regression coefficients represent the SD unit change in lipoprotein measure for a 1 SD increase in PA variable. In the change models, activity intensities were modelled as the z-score of follow-up minus baseline activity measure. We used principal component analysis (PCA) to estimate the effective number of independent tests to use for multiple testing correction. The rationale for this method has been described previously,[25] and applied in a number of metabolic profile studies.[26,27] Using z-scores of the 57 lipoprotein measures, we calculated that 5 principal components explained >95% of the variance. Hence, our Bonferroni-corrected threshold for assessing associations is 0.05/5 = 0.01 (i.e., p <0.01). All analyses were conducted using R version 3.6.3 (R Foundation for Statistical

Computing, Vienna, Austria). 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 PCA analysis with **factoextra** (1.0.6) and 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

Our analytical sample for the prospective analyses comprised 762 children with complete data and at least four valid days of accelerometer measurements (49.6% girls) (Figure 1). The average interval between baseline and follow-up accelerometer testing was 46.4 weeks. 52.9% of the sample had seven valid days of accelerometer data. Of the 762 children included in the prospective analyses, 720 had at least four days of valid follow-up accelerometer data, hence were included in the change score analyses. Correlations between the baseline and follow-up activity intensity measures were moderate (Pearson's r = 0.52 for VPA, 0.53 for MPA, 0.57 for LPA, and 0.53 for sedentary time; p <0.001 for each). Descriptive information for the analytical sample is given in Table 1. Means and SDs for the NMR lipoprotein measures are provided in Supplementary Material Table 1.

Those children not included in the analytical sample tended to be slightly older, shorter, and typically recorded lower daily minutes of VPA, MPA, LPA, and were less likely to achieve the recommended daily amount (≥60 minutes) of MVPA (Supplementary Material Table 2). A lower proportion were overweight or obese.

3.2. Vigorous-intensity physical activity

In the analysis of VPA, a 1 SD (16.2 $min \cdot d^{-1}$) increment was associated with a number of lipoprotein measures (Figure 2). There were moderate to strong inverse associations with almost all measures of the VLDL particles, excluding the VLDL S subclass (e.g., $–1.32 \times 10^{-1}$ $nmol \cdot L^{-1}$ or –0.13 SD; CI = –0.19, –0.06; p <0.001 for VLDL L1 particle number). The association magnitudes for the measures of particle numbers, cholesterol concentrations, and triglycerides concentrations for the VLDL subclasses decreased in strength from largest to smallest particles. Higher levels of VPA seemed to be inversely associated with measures of low-density lipoprotein (LDL) subclasses, though the associations were weaker than for the larger CM and VLDL particles. However, the associations with triglycerides concentrations of LDL S and LDL VS were markedly stronger than for the other subclasses (e.g., $–3.95 \times 10^{-4}$ $mmol \cdot L^{-1}$ or –0.14 SD; CI = –0.21, –0.08; p <0.001 for LDL VS triglycerides concentration). Directions of association with the HDL subclass measures tended to differ dependent on particle size, though most associations weren't robust. However, associations with the triglycerides concentrations of the two smallest HDL subclasses were comparatively stronger (e.g., $–3.33 \times 10^{-4}$ $mmol \cdot L^{-1}$ or –0.13 SD; CI = –0.19, –0.07; p <0.001 for HDL VS triglycerides concentration). Higher VPA was positively associated with the average diameter of LDL, and inversely with total triglycerides concentration and non-HDL cholesterol concentration. Including waist circumference as an additional covariate did not alter the overall pattern of associations compared to the standard model. Attenuation for individual measures

was small to moderate (absolute difference range = 0.00–0.05 SD) and tended to be greater for the larger subclasses (Supplementary Material Table 3).
Associations with change in VPA (mean = –4.7 min·d^{-1}) were weak (Supplementary Material Figure 1). Of these, the subclasses with which the associations were more pronounced were VLDL M, VLDL S, and LDL L, and more so for triglycerides concentrations. A 1 SD (14.9 min·d^{-1}) increase in activity being associated with lower levels of these measures (e.g., –8.50 x 10^{-4} mmol·L^{-1} or –0.05 SD; CI = –0.13, 0.03; p = 0.19 for LDL L triglycerides concentration). Adjustment for adiposity had a negligible effect on associations (absolute difference range = 0.00–0.01 SD). Results in Supplementary Material Table 7.

3.3. Moderate-intensity physical activity
The pattern of associations between a 1 SD (12.8 min·d^{-1}) increase in MPA and the lipoprotein profile was broadly similar to the pattern for a 1 SD increase in VPA (Figure 3). However, the magnitudes were smaller for all but four of the 57 lipoprotein measures. The association directions of the VLDL measures were the same as for VPA, except for the particle number and cholesterol concentration of the VLDL S subclass. Associations with measures of the LDL subclasses tended to be weak to moderate, though the triglycerides concentrations of LDL S and LDL VS were again more marked (e.g., –3.66 x 10^{-4} mmol·L^{-1} or –0.13 SD; CI = –0.21, –0.05; p = 0.002 for LDL VS triglycerides concentration). The directions were all inverse, except for the LDL L subclass. Almost all associations between MPA and the HDL measures were weaker than in the VPA analysis, and a number were almost zero. Adjusting the standard model for waist circumference had a limited effect (absolute difference range = 0.00–0.03 SD). The direction of association changed for three subclass measures, but all were close to zero before and after adjustment for waist circumference, and the difference in effect size ≤0.01 SD (Supplementary Material Table 4).
The overall pattern of associations between a 1 SD (12.1 min·d^{-1}) change in MPA (mean = –4.9 min·d^{-1}) was similar to that of the VPA model (Supplementary Material Figure 2), though many of the individual associations were stronger (e.g., –4.00 x 10^{-3} mmol·L^{-1} or –0.08 SD; CI = –0.13, –0.02; p = 0.01 for VLDL S cholesterol concentration). Associations with the triglycerides concentrations of the larger HDL subclasses were moderate, as was the association with average LDL particle diameter. Attenuation having adjusted for adiposity was limited (absolute difference range = 0.00–0.01 SD). Results in Supplementary Material Table 8.

3.4. Light-intensity physical activity
In contrast to the VPA and MPA analyses, the associations between a 1 SD (36.7 min·d^{-1}) increment in LPA and measures of VLDL particles were generally more modest (Figure 4). Individual associations with some specific subclasses were very weak, such as those with VLDL L3 and VLDL M. Conversely, associations with VLDL S were stronger than with most other VLDL subclasses (e.g., 1.68 x 10^{-3} mmol·L^{-1} or 0.03 SD; CI = –0.04, 0.10; p = 0.37 for VLDL S cholesterol concentration). Almost all of the LDL measures were positively associated with LPA. The associations with cholesterol concentrations and particle numbers of the two larger LDL subclasses were quite robust (e.g., 1.27 x 10^{-2} mmol·L^{-1} or 0.06 SD; CI = 0.00, 0.11, p = 0.04 for LDL M cholesterol concentration). The distinct inverse associations with

triglycerides concentrations in LDL S and LDL VS remained but were weaker than in other analyses, and with LDL average particle diameter it was almost zero. The divergent directions of association between the particle numbers and cholesterol concentrations of the HDL subclasses were apparent. Except for the measures of triglycerides concentration, all associations were stronger than, and in the opposite directions to, those in the VPA analysis. Adjustment for waist circumference had little effect (≤0.01 SD absolute difference for each measure). The association direction changed for VLDL L3, but the effect size was almost zero both before and after adjustment (Supplementary Material Table 5).
The associations between a 1 SD (33.3 $min \cdot d^{-1}$) change in LPA level were not robust (Supplementary Material Figure 3), nor did adjustment for waist circumference have much impact (absolute difference range = 0.00–0.02 SD). Results in Supplementary Material Table 9.

3.5. Sedentary time

The strengths of associations for a 1 SD increment (57.5 $min \cdot d^{-1}$) in sedentary time tended to differ dependent on lipoprotein class; associations with VLDL particles typically being stronger than with LDL and HDL particles (Figure 5). All associations with the VLDL measures, except VLDL S, were in the opposite direction to those in the VPA analysis (e.g., 1.22 x 10^{-1} $nmol \cdot L^{-1}$ or 0.12 SD; CI = 0.03, 0.21; $p = 0.01$ for VLDL L1 particle number). Though the effect magnitudes were smaller, the pattern of effect sizes decreasing from largest to smallest particles was reproduced. Association directions between time spent sedentary and the measures of particle numbers and cholesterol concentrations of the LDL and HDL subclasses tended to differ dependent on subclass particle size, though the majority of individual effects were weak to moderate. Again, the triglycerides concentrations of the LDL S and LDL VS subclasses were distinct. There were also moderate, positive associations with the triglycerides concentrations of the largest and smallest HDL subclasses, and with the total triglycerides concentration. Attenuation having adjusted for waist circumference was small (absolute difference range = 0.00–0.03 SD). The direction of association changed for one measure (LDL S cholesterol concentration), though the effect size was almost zero before and after adjustment (Supplementary Material Table 6).
Change in sedentary time (mean = 28.3 $min \cdot d^{-1}$) was weakly associated with the lipoprotein profile (Supplementary Material Figure 4). The association directions between a 1 SD (52.8 $min \cdot d^{-1}$) change in sedentary time tended to be opposite to those with MPA, but less robust (e.g., 2.97 x 10^{-3} $mmol \cdot L^{-1}$ or 0.06 SD; CI = –0.01, 0.13; $p = 0.11$ for VLDL S cholesterol concentration). The attenuation of associations for waist circumference was limited (absolute difference range = 0.00–0.03 SD). Results in Supplementary Material Table 10.

4. Discussion

We aimed to investigate the prospective associations of objectively measured PA and sedentary time with a comprehensive lipoprotein profile across the school year. We found that, broadly speaking, higher levels and higher intensities of PA are associated with an apparently favourable lipoprotein phenotype, whereas greater time spent sedentary seems detrimental. Effect sizes were modest, however, which suggests that the influence of PA on individual

measures is limited. Associations with changes in PA or sedentary time were weak, which may potentially be due to the small overall changes in this active population.

Previous studies have reported similar beneficial lipoprotein profiles with higher levels of PA. In a study of device-measured physical activity and a comprehensive metabolic profile in adolescents, the authors showed stronger associations with MVPA than sedentary time.[10] However, the associations between MVPA and the metabolic profile in the cross-sectional analysis did not differ based on previous activity levels, which the authors interpreted as the effect of PA being dependent on recent engagement. In our results, change in level of physical activity were weakly associated with lipoprotein measures compared to the prospective analysis, which might indicate that previous activity levels are important. However, though the activity levels of our children seem to have declined and sedentary time increased between the two measurement occasions, the absolute level of PA remained high (mean MVPA = 68.0 $min \cdot d^{-1}$ at follow-up). If there is a ceiling level above which PA has diminished and/or limited influence on lipoprotein metabolism, then given that the majority of our children had consistently high levels of PA (mean ΔMVPA = –9.5 $min \cdot d^{-1}$), this would suggest that any change in PA have only negligible effects in our cohort. It could also be that unmeasured behaviours strongly associated with lipoprotein metabolism, like diet, were consistent over time even though the PA level changed.

A study in adults compared those who self-reported their level of PA as "active" to those who reported being "inactive" on two occasions at least five years apart.[11] Being consistently active was inversely associated with the particle concentrations of all ApoB-containing lipoprotein subclasses, a number of measures of subclass triglycerides concentration, and positively associated with both the particle concentrations of and cholesterol concentrations of the larger HDL subclasses. Many of the associations were more pronounced compared to those in our results, which could be for a number of reasons. Firstly, the effect sizes reported for each lipoprotein measure were expressed using the SD unit *difference* in leisure-time PA between those categorised as active or inactive, which are likely to be greater than the SD unit *increment*s of the continuous PA intensity measures used in our study. The participants tended to be older and the time elapsed between the baseline and follow-up measurements far greater (e.g., 16 years in one of the included cohorts) than in our study. Thus, there is more likely to be greater variation in metabolism due to the accumulation of comorbidities with time and age. Activity levels of the included participants were likely more consistent across the two time points given that consistent level of activity was a criterion for inclusion.

Physical activity at either moderate-intensity or vigorous-intensity share a very similar pattern of associations with the lipoprotein measures analysed. These effects were typically stronger with the larger, triglyceride-enriched VLDLs, but also the triglycerides concentrations of the smallest LDL and HDL particles. These same measures were those most strongly associated with time spent sedentary, though in the opposite direction. This may reflect differences in fasting triglycerides as a result of differences in energy expenditure,[28] to which exercise intensity is related, or differences in energy intake [29] or nutrient composition of meals.[30] Recent evidence suggests that the well-recognised causal effect of LDL on atherosclerotic cardiovascular disease (ASCVD) risk [31,32] may not be a result of the lipid mass that the particles carry,[33] but of the concentration of particles in the circulation,[34,35] which can be quantified by measuring the apolipoprotein B (ApoB) concentration. Apolipoprotein B is the primary

apolipoprotein of chylomicron, VLDL, and LDL particles, and likely the causal trait that enables the lipids carried by these lipoproteins to exert their influence on CVD risk.[36,37] Moreover, all ApoB-containing lipoproteins up to 70 nm diameter, which includes triglyceride-rich and cholesterol-rich chylomicron and VLDL remnant particles, can penetrate the arterial intima and are thought to be similarly atherogenic.[33,38] Our results demonstrate relatively stronger associations of PA and sedentary time with VLDL particles, compared to the LDLs. Given that the circulating concentration (number) of particles likely determines the probability of them entering and being retained in the intima,[39] any cardioprotective effects of increased PA or reduced time spent sedentary seem likely expressed through the metabolism of these larger apolipoprotein B-containing subclasses. Unexpectedly, LPA was positively associated with a number of LDL subclass measures, which has previously been reported and may be a consequence of some sedentary activities being misclassified as LPA.[40]

Substantial structural, compositional, and functional heterogeneity exists between HDL particles. In addition to reverse cholesterol transport (RCT), HDLs participate in antioxidative, anti-inflammatory, and anti-infectious activities, among others, and this assortment of biological functions seems to be mediated by different particle subpopulations.[41] Surprisingly, in our study particle numbers and cholesterol concentrations of the HDL subclasses were directionally consistent between VPA and sedentary time, suggesting a shift towards larger HDL particles and increased serum HDL cholesterol. However, the associations were weak in both analyses and negligible in the MPA model, so may not be true effects. If true, it is challenging to provide a mechanistic explanation for this apparent paradox contingent on energy expenditure alone, especially given that the associations were in the reverse direction and quite robust in the LPA analyses. Instead, these results may reflect the poor characterisation by particle number or lipid mass of HDL physicochemical and functional heterogeneity,[41] and our incomplete understanding of how HDL function changes with either total PA or PA of different intensities. Historically, that higher HDL cholesterol levels are associated with higher levels of PA and lower CVD indicated a potential means through which PA exerts its cardioprotective effect.[42] However, recent evidence from clinical trials [43] and Mendelian randomisation [44,45] suggests that a direct causal effect of HDL cholesterol level on CVD is unlikely. Furthermore, there is preliminary evidence that exercise benefits some HDL attributes independent of changes in HDL cholesterol.[46] Consequently, greater research effort has been directed to quantifying HDL functionality, its influence on CVD risk, and the effects of PA on HDL beyond the traditional lipid profile.[47,48] The generally modest associations with HDL measures in our results suggest the cardioprotective effects of PA are either inadequately characterised by measures of particle number and lipid load, or alternatively, indicative of limited metabolic perturbation in our young, healthy cohort.

In our analyses, associations were moderately attenuated having adjusted for waist circumference, which suggests an independent effect of PA and sedentary time on our lipoprotein measures. Adiposity has been shown to be causally associated with the lipoprotein profile in young adults,[49] and that it mediates a proportion of the beneficial effects of MVPA on lipid measures.[8] There is also robust evidence that higher levels of adiposity are causal for lower total PA, MVPA, and increased time spent sedentary in children, and these associations are much stronger when adiposity is modelled as the exposure rather than the outcome.[50] Considered in the context of these studies, our results suggest that increasing VPA or MPA

could benefit the lipoprotein profile, but an intervention that achieves a concomitant reduction in, or prevents increases in, adiposity would likely be synergistic.

4.1. Strengths and limitations

The use of a targeted metabolomics platform enabled us to investigate a variety of lipoprotein measures, providing a more nuanced description of the associations with PA and sedentary time than could be achieved with a standard lipid profile. We modelled the activity measures both prospectively and as change scores, adjusting for the baseline value of each respective lipoprotein measure, which enabled us to examine the temporality of associations. The blood samples were drawn with the children having fasted and at a consistent time of day, limiting potential variability due to dietary intake and daily activity. We had a high level of participant compliance with the PA assessment, though we acknowledge that one week of PA assessed at two time points may not be fully representative of behavioural variability over many months. Furthermore, substantial intraindividual variation has been reported when measuring children's PA using accelerometers over a 1-year period such that the true regression coefficients may be underestimated by up to 50%.[51] Device-based measures of PA are less prone to the biases typical of self-report activity data, and the devices used were triaxial, which have been shown to capture more activity than uniaxial accelerometers.[52]

There were also several limitations. Our data are observational hence we cannot exclude unmeasured confounding from biasing our effect estimates. Importantly, we did not have dietary information for our children. Nonfasted samples are considered more representative of the predominant metabolic state.[53] Our use of fasted samples precluded us from investigating the potential for PA to mitigate the postprandial rise in triglycerides and commensurate rise in circulating chylomicrons, which are thought to contribute to increased atherosclerotic risk.[28] The children that participated in the ASK study were young and the period over which they were followed short, so metabolic variation is likely limited. It would be instructive to follow them for a longer period or as they transition through adolescence into adulthood to observe whether the potentially beneficial effects of PA augment with age. Our cohort are homogenous and also highly active in comparison to the other adolescents both within Norway and globally,[54] which likely limits the generalisability of our findings to other populations.

4.2. Conclusion

Our study shows that more time spent being physically active—especially at higher intensities—is prospectively associated with a favourable lipoprotein profile, whereas more time spent sedentary appears to be detrimental. These associations are largely independent of adiposity. If causal, the mechanisms that drive these benefits are likely due to alterations in triglycerides metabolism, which may explain the typically inconclusive results of PA studies that only examine the standard lipid profile.

References

1 Roth GA, Abate D, Abate KH, *et al.* Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2018; **392**: 1736–88.

2 Kraus WE, Powell KE, Haskell WL, *et al.* Physical Activity, All-Cause and Cardiovascular Mortality, and Cardiovascular Disease. *Med Sci Sports Exerc* 2019; **51**: 1270–1281.

3 Arnett Donna K., Blumenthal Roger S., Albert Michelle A., *et al.* 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* 2019; **140**: e596–646.

4 U.S. Department of Health and Human Services. Physical activity guidelines for Americans, 2nd edition. Washington, D.C.: U.S. Department of Health and Human Services, 2018.

5 Cristi-Montero C, Chillón P, Labayen I, *et al.* Cardiometabolic risk through an integrative classification combining physical activity and sedentary behavior in European adolescents: HELENA study. *J Sport Health Sci* 2019; **8**: 55–62.

6 Tarp J, Child A, White T, *et al.* Physical activity intensity, bout-duration, and cardiometabolic risk markers in children and adolescents. *Int J Obes* 2018; **42**: 1639–50.

7 Poitras VJ, Gray CE, Borghese MM, *et al.* Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Appl Physiol Nutr Metab* 2016; **41**: S197–239.

8 Tarp J, Bugge A, Andersen LB, *et al.* Does adiposity mediate the relationship between physical activity and biological risk factors in youth?: a cross-sectional study from the International Children's Accelerometry Database (ICAD). *Int J Obes* 2018; **42**: 671–8.

9 Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ Cardiovasc Genet* 2015; **8**: 192–206.

10 Bell JA, Hamer M, Richmond RC, Timpson NJ, Carslake D, Smith GD. Associations of device-measured physical activity across adolescence with metabolic traits: Prospective cohort study. *PLOS Med* 2018; **15**: e1002649.

11 Kujala UM, Makinen V-P, Heinonen I, *et al.* Long-term Leisure-time Physical Activity and Serum Metabolome. *Circulation* 2013; **127**: 340–8.

12 Jones PR, Rajalahti T, Resaland GK, *et al.* Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis* 2019; **288**: 186–93.

13 Resaland GK, Aadland E, Moe VF, *et al.* Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. *Prev Med* 2016; **91**: 322–8.

14 World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* 2013; **310**: 2191–4.

15 Esliger DW, Copeland JL, Barnes JD, Tremblay MS. Standardizing and Optimizing the Use of Accelerometer Data for Free-Living Physical Activity Monitoring. *J Phys Act Health* 2005; **2**: 366–83.

16 Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. *J Sports Sci* 2008; **26**: 1557–65.

17 Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of Accelerometer Cut Points for Predicting Activity Intensity in Youth: *Med Sci Sports Exerc* 2011; **43**: 1360–8.

18 Okazaki M. Identification of Unique Lipoprotein Subclasses for Visceral Obesity by Component Analysis of Cholesterol Profile in High-Performance Liquid Chromatography. *Arterioscler Thromb Vasc Biol* 2005; **25**: 578–84.

19 Okazaki M, Yamashita S. Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using "Spherical Particle Model". *J Oleo Sci* 2016; **65**: 265–82.

20 Lin C, Rajalahti T, Mjøs SA, Kvalheim OM. Predictive associations between serum fatty acids and lipoproteins in healthy non-obese Norwegians: implications for cardiovascular health. *Metabolomics* 2015; **12**: 6.

21 Kvalheim OM, Arneberg R, Grung B, Rajalahti T. Determination of optimum number of components in partial least squares regression from distributions of the root-mean-squared error obtained by Monte Carlo resampling. *J Chemom* 2018; **32**: e2993.

22 Nanjee MN, Brinton EA. Very Small Apolipoprotein A-I-containing Particles from Human Plasma: Isolation and Quantification by High-Performance Size-Exclusion Chromatography. *Clin Chem* 2000; **46**: 207–23.

23 Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–94.

24 Carel J-C, Léger J. Precocious Puberty. *N Engl J Med* 2008; **358**: 12.

25 Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008; **32**: 361–9.

26 Ferreira DLS, Williams DM, Kangas AJ, *et al.* Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. *PLOS Med* 2017; **14**: e1002376.

27 Kujala UM, Vaara JP, Kainulainen H, Vasankari T, Vaara E, Kyröläinen H. Associations of Aerobic Fitness and Maximal Muscular Strength With Metabolites in Young Men. *JAMA Netw Open* 2019; **2**: e198265–e198265.

28 Peddie MC, Rehrer NJ, Perry TL. Physical activity and postprandial lipidemia: Are energy expenditure and lipoprotein lipase activity the real modulators of the positive effect? *Prog Lipid Res* 2012; **51**: 11–22.

29 Pearson N, Biddle SJH. Sedentary Behavior and Dietary Intake in Children, Adolescents, and Adults: A Systematic Review. *Am J Prev Med* 2011; **41**: 178–88.

30 Dunton GF, O'Connor SG, Belcher BR, Maher JP, Schembre SM. Objectively-Measured Physical Activity and Sedentary Time are Differentially Related to Dietary Fat and Carbohydrate Intake in Children. *Front Public Health* 2018; **6**: 198.

31 Borén J, Chapman MJ, Krauss RM, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2020; published online Feb 13. DOI:10.1093/eurheartj/ehz962.

32 Ference BA, Ginsberg HN, Graham I, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017; **38**: 2459–72.

33 Ference BA, Kastelein JJP, Ray KK, *et al.* Association of Triglyceride-Lowering LPL Variants and LDL-C–Lowering LDLR Variants With Risk of Coronary Heart Disease. *JAMA* 2019; **321**: 364–73.

34 Ference BA, Kastelein JJP, Ginsberg HN, *et al.* Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk. *JAMA* 2017; **318**: 947–56.

35 Sniderman AD, Couture P, Martin SS, *et al.* Hypertriglyceridemia and cardiovascular risk: a cautionary note about metabolic confounding. *J Lipid Res* 2018; **59**: 1266–75.

36 Richardson TG, Sanderson E, Palmer TM, *et al.* Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. *PLOS Med* 2020; **17**: e1003062.

37 Ala-Korpela M. The culprit is the carrier, not the loads: cholesterol, triglycerides and apolipoprotein B in atherosclerosis and coronary heart disease. *Int J Epidemiol* 2019; **48**: 1389–92.

38 Chait A, Ginsberg HN, Vaisar T, Heinecke JW, Goldberg IJ, Bornfeldt KE. Remnants of the Triglyceride-Rich Lipoproteins, Diabetes, and Cardiovascular Disease. *Diabetes* 2020; **69**: 508–16.

39 Borén J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidol* 2016; **27**: 473–483.

40 Hansen BH, Anderssen SA, Andersen LB, *et al.* Cross-Sectional Associations of Reallocating Time Between Sedentary and Active Behaviours on Cardiometabolic Risk Factors in Young People: An International Children's Accelerometry Database (ICAD) Analysis. *Sports Med* 2018; **48**: 2401–12.

41 Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med* 2011; **17**: 594–603.

42 März W, Kleber ME, Scharnagl H, *et al.* HDL cholesterol: reappraisal of its clinical relevance. *Clin Res Cardiol* 2017; **106**: 663–75.

43 Lincoff AM, Nicholls SJ, Riesmeyer JS, *et al.* Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. *N Engl J Med* 2017; **376**: 1933–42.

44 Holmes MV, Asselbergs FW, Palmer TM, *et al.* Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J* 2014; **36**: 539–50.

45 Voight BF, Peloso GM, Orho-Melander M, *et al.* Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *The Lancet* 2012; **380**: 572–80.

46 Ruiz-Ramie JJ, Barber JL, Sarzynski MA. Effects of exercise on HDL functionality. *Curr Opin Lipidol* 2019; **30**: 16–23.

47 Allard-Ratick MP, Kindya BR, Khambhati J, *et al.* HDL: Fact, fiction, or function? HDL cholesterol and cardiovascular risk. *Eur J Prev Cardiol* 2019; : 2047487319848214.

48 Jomard A, Osto E. High Density Lipoproteins: Metabolism, Function, and Therapeutic Potential. *Front Cardiovasc Med* 2020; **7**: 39.

49 Würtz P, Wang Q, Kangas AJ, *et al.* Metabolic Signatures of Adiposity in Young Adults: Mendelian Randomization Analysis and Effects of Weight Change. *PLoS Med* 2014; **11**: e1001765.

50 Richmond RC, Davey Smith G, Ness AR, den Hoed M, McMahon G, Timpson NJ. Assessing Causality in the Association between Child Adiposity and Physical Activity Levels: A Mendelian Randomization Analysis. *PLoS Med* 2014; **11**: e1001618.

51 Mattocks C, Leary S, Ness A, *et al.* Intraindividual Variation of Objectively Measured Physical Activity in Children. *Med Sci Sports Exerc* 2007; **39**: 622–9.

52 Sagelv EH, Ekelund U, Pedersen S, *et al.* Physical activity levels in adults and elderly from triaxial and uniaxial accelerometry. The Tromsø Study. *PLOS ONE* 2019; **14**: e0225670.

53 Nordestgaard BG. A Test in Context: Lipid Profile, Fasting Versus Nonfasting. *J Am Coll Cardiol* 2017; **70**: 1637–46.

54 Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1·6 million participants. *Lancet Child Adolesc Health* 2020; **4**: 23–35.

Conflict of interest
The Authors declare that there is no conflict of interest.

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Authors' contributions
PRJ, GKR, EA, JS-J, SAA, TFB, TR, OMK, and UE contributed to the conception and design of the work. TR, GKR, EA, TFB, and TA contributed to data acquisition. PRJ, TR, TA, and OMK contributed to data analysis. PRJ, 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.

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Figure legends

Figure 1. Flow of participants through the study indicating number of children that had valid data available

The final analytical sample included those children that had valid data for all baseline variables and blood samples at follow-up.

Figure 2. Associations between baseline vigorous-intensity physical activity (VPA) and follow-up lipoprotein measures

The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 3. Associations between baseline moderate-intensity physical activity (MPA) and follow-up lipoprotein measures

The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 4. Associations between baseline light-intensity physical activity (LPA) and follow-up lipoprotein measures

The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 5. Associations between baseline sedentary time and follow-up lipoprotein measures

The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment in activity. The models are adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 1. Characteristics of children included in the analytical sample

Characteristic	*n* (%)	Mean (SD)
Baseline	762	
Age (years)		10.0 (0.3)
Sex		
Girls	378 (49.6)	
Boys	384 (50.4)	
Anthropometry		
Height (m)		143.1 (6.7)
Weight (kg)		37.2 (8.1)
BMI ($kg \cdot m^{-2}$)		18.1 (3.0)
≥25	174 (22.8)	
≥30	38 (5.0)	
Waist circumference (cm)		62.1 (7.6)
Parents' education		
Upper secondary school	241 (31.6)	
<4 years college/university	229 (30.1)	
≥4 years college/university	292 (38.3)	
Tanner stage		
Stage 1	417 (54.7)	
Stage 2	297 (39.0)	
Stage ≥3	48 (6.3)	
Physical activity		
VPA ($min \cdot d^{-1}$)		32.2 (16.2)
MPA ($min \cdot d^{-1}$)		45.1 (12.8)
LPA ($min \cdot d^{-1}$)		235.2 (36.7)
SED ($min \cdot d^{-1}$)		466.5 (57.5)
MVPA ≥60 ($min \cdot d^{-1}$)	563 (73.9)	
Lipid profile		
TC ($mmol \cdot L^{-1}$)		4.5 (0.7)
LDL-C ($mmol \cdot L^{-1}$)		2.5 (0.6)
HDL-C ($mmol \cdot L^{-1}$)		1.6 (0.3)
TG ($mmol \cdot L^{-1}$)[a]		0.7 [0.5, 0.9]
Follow-up	720	

Physical activity		
ΔVPA_{t2-t1} (min·d^{-1})		–4.7 (14.9)
ΔMPA_{t2-t1} (min·d^{-1})		–4.9 (12.1)
ΔLPA_{t2-t1} (min·d^{-1})		–14.3 (33.3)
ΔSED_{t2-t1} (min·d^{-1})		28.3 (52.8)
MVPA ≥60 (min·d^{-1})	451 (62.6)	
Lipid profile		
TC (mmol·L^{-1})		4.5 (0.6)
LDL-C (mmol·L^{-1})		2.6 (0.6)
HDL-C (mmol·L^{-1})		1.6 (0.3)
TG (mmol·L^{-1})[a]		0.6 [0.5, 0.9]

[a]Median [IQR].
Abbreviations: BMI = body mass index; HDL-C = high-density lipoprotein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; LPA = light-intensity physical activity; MPA = moderate-intensity physical activity; MVPA = moderate- to vigorous-intensity physical activity; SD = standard deviation; SED = sedentary time; TC = total cholesterol; TG = triglycerides; VPA = vigorous-intensity physical activity.

Figure 1.

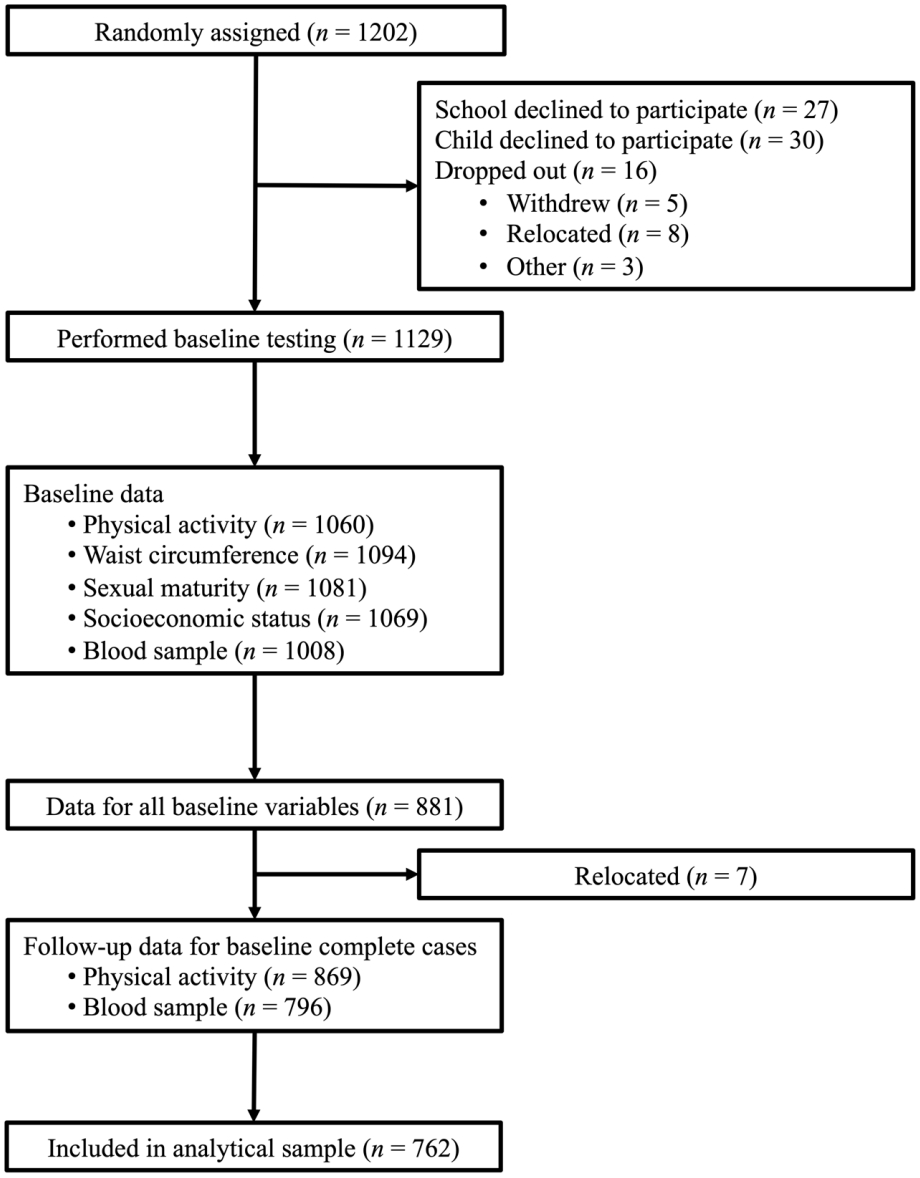
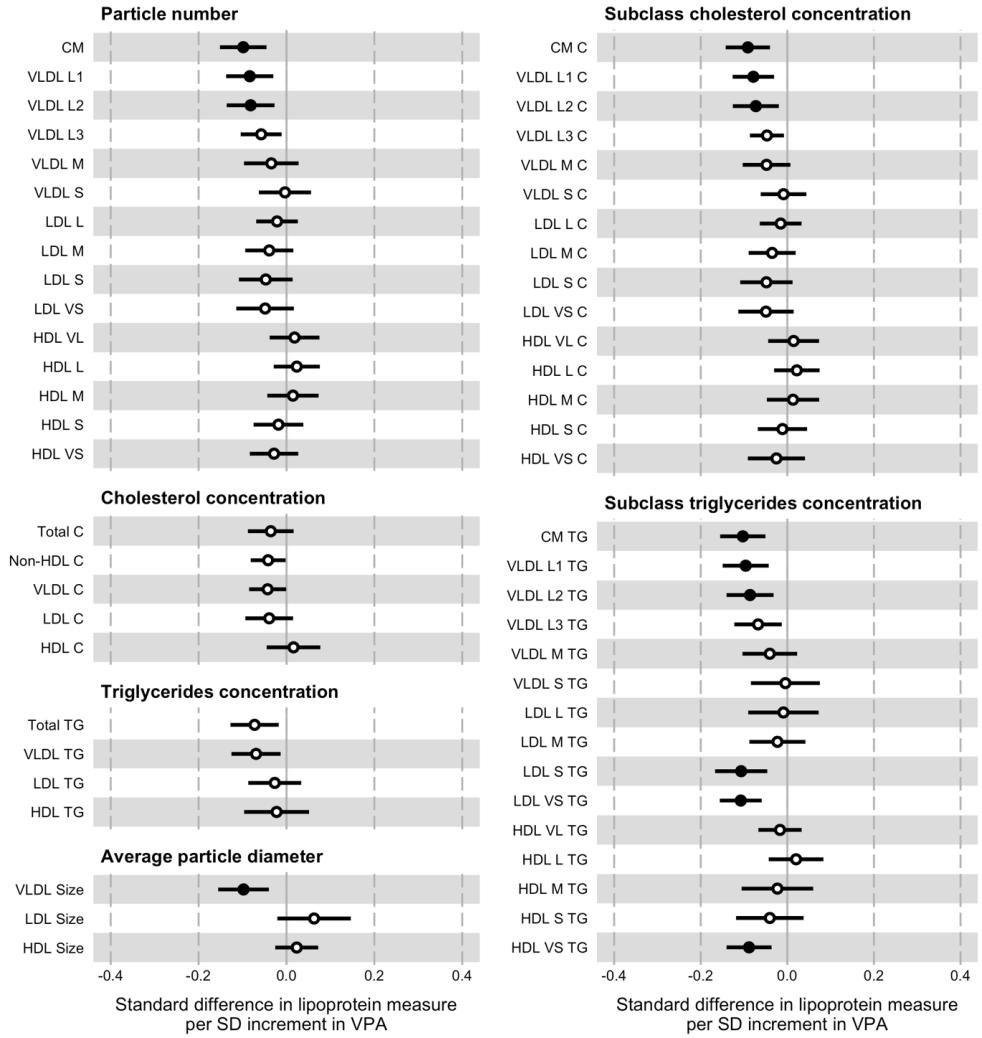

Randomly assigned (n = 1202)
School declined to participate (n = 27)
Child declined to participate (n = 30)
Dropped out (n = 16)
• Withdrew (n = 5)
• Relocated (n = 8)
• Other (n = 3)
Performed baseline testing (n = 1129)
Baseline data
• Physical activity (n = 1060)
• Waist circumference (n = 1094)
• Sexual maturity (n = 1081)
• Socioeconomic status (n = 1069)
• Blood sample (n = 1008)
Data for all baseline variables (n = 881)
Relocated (n = 7)
Follow-up data for baseline complete cases
• Physical activity (n = 869)
• Blood sample (n = 796)
Included in analytical sample (n = 762)

Figure 2.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VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.4
-0.2
0.0
0.2
0.4
Standard difference in lipoprotein measure per SD increment in VPA
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
HDL S TG
HDL VS TG
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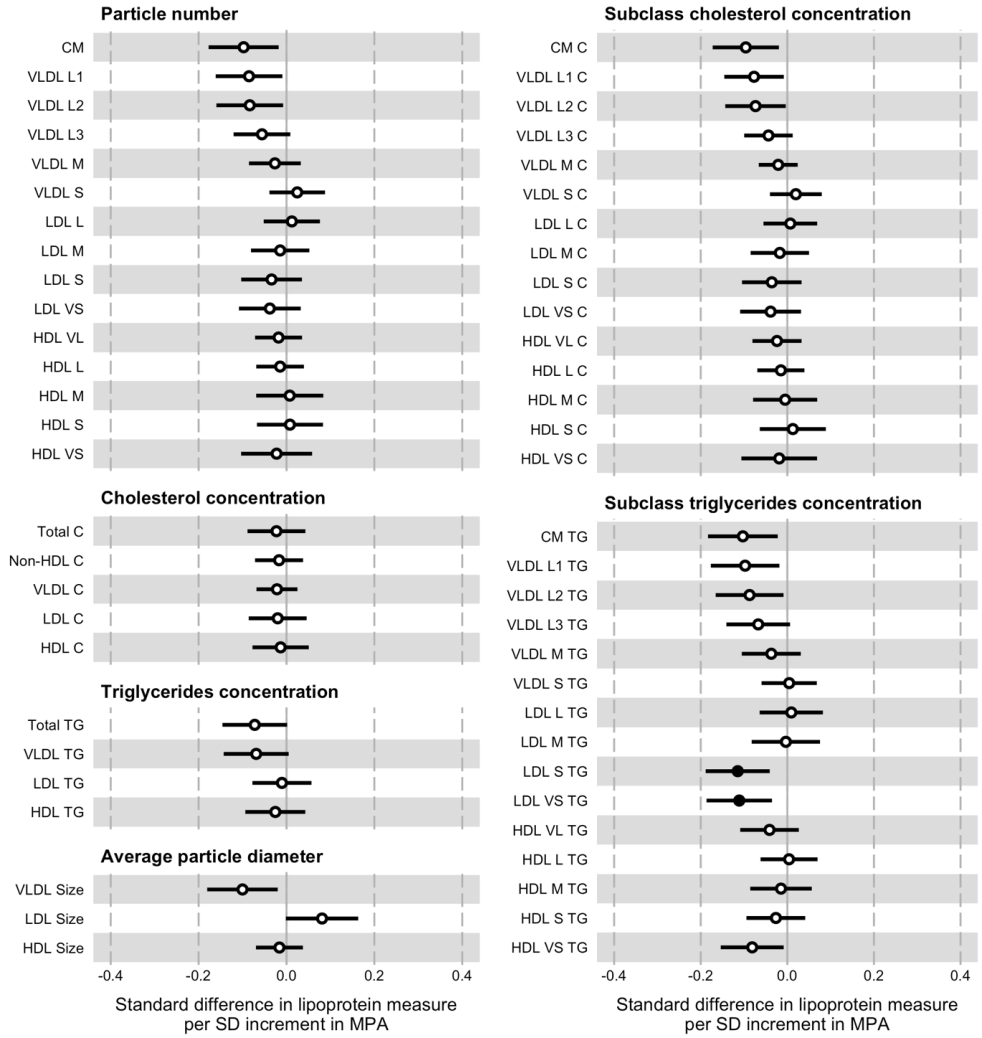
Standard difference in lipoprotein measure per SD increment in VPA

Figure 3.


Particle number
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VLDL L2
VLDL L3
VLDL M
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LDL M
LDL S
LDL VS
HDL VL
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HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.4
-0.2
0.0
0.2
0.4
Standard difference in lipoprotein measure per SD increment in MPA
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
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0.2
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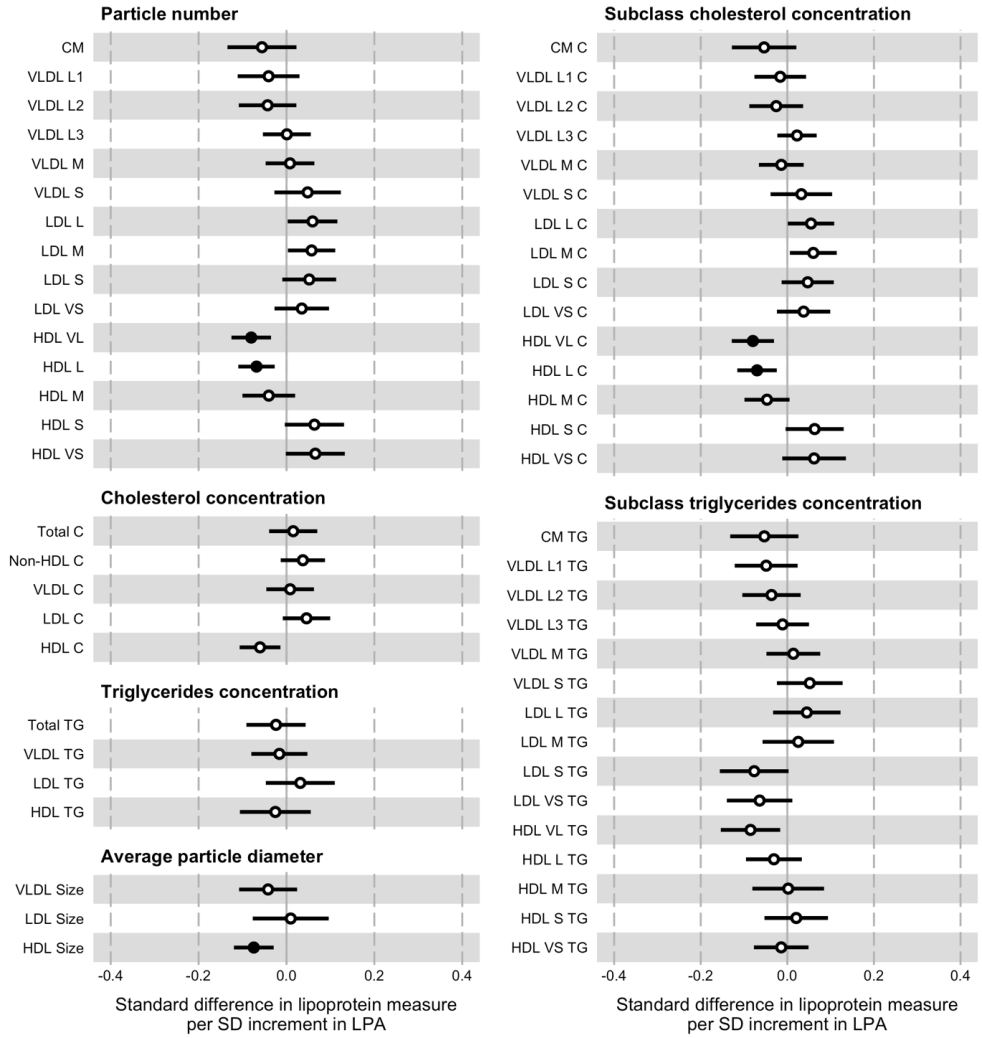
Standard difference in lipoprotein measure per SD increment in MPA

Figure 4.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HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.4
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0.0
0.2
0.4
Standard difference in lipoprotein measure per SD increment in LPA
Subclass cholesterol concentration
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VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
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VLDL L2 TG
VLDL L3 TG
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VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
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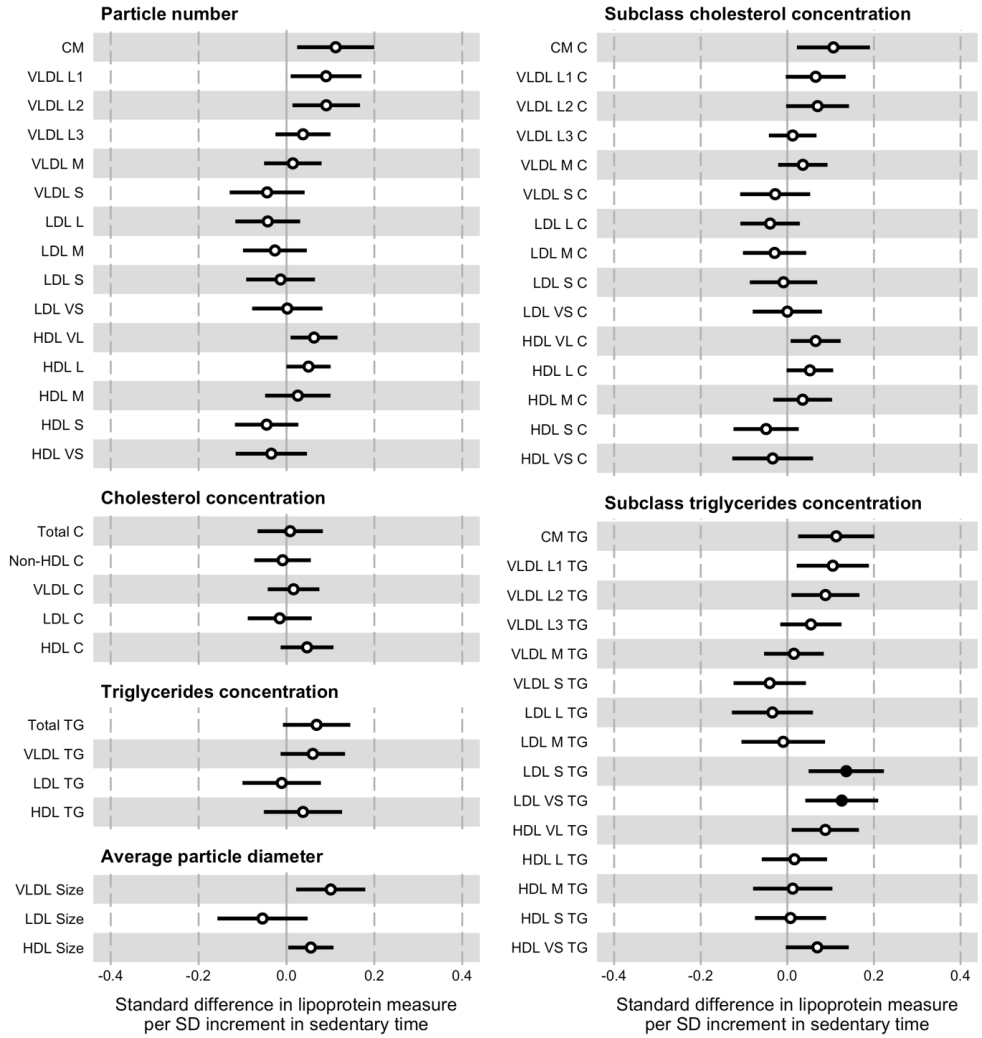
0.4
Standard difference in lipoprotein measure per SD increment in LPA

Figure 5.


Particle number
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VLDL L1
VLDL L2
VLDL L3
VLDL M
VLDL S
LDL L
LDL M
LDL S
LDL VS
HDL VL
HDL L
HDL M
HDL S
HDL VS
Cholesterol concentration
Total C
Non-HDL C
VLDL C
LDL C
HDL C
Triglycerides concentration
Total TG
VLDL TG
LDL TG
HDL TG
Average particle diameter
VLDL Size
LDL Size
HDL Size
-0.4
-0.2
0.0
0.2
0.4
Standard difference in lipoprotein measure per SD increment in sedentary time
Subclass cholesterol concentration
CM C
VLDL L1 C
VLDL L2 C
VLDL L3 C
VLDL M C
VLDL S C
LDL L C
LDL M C
LDL S C
LDL VS C
HDL VL C
HDL L C
HDL M C
HDL S C
HDL VS C
Subclass triglycerides concentration
CM TG
VLDL L1 TG
VLDL L2 TG
VLDL L3 TG
VLDL M TG
VLDL S TG
LDL L TG
LDL M TG
LDL S TG
LDL VS TG
HDL VL TG
HDL L TG
HDL M TG
HDL S TG
HDL VS TG
-0.4
-0.2
0.0
0.2
0.4
Standard difference in lipoprotein measure per SD increment in sedentary time

Paper III supplementary material

Supplementary methods

Blood samples

Serum was drawn from an antecubital vein and obtained according to a standard protocol consisting of the following steps: i) Blood plasma was collected in 5 ml VACUETTE Serum Gel with Activator blood collection tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria). ii) The tubes were carefully inverted five times and placed vertically for coagulation. iii) After 30 minutes, the samples were centrifuged at 2000 G for ten minutes. Serum was then visually inspected for residue and centrifugation was repeated if residue was present. iv) The tubes were kept in a refrigerator at 4°C before pipetting 0.5 ml into cryo tubes. v) The cryo tubes were then stored in a freezer at –20°C for up to 2 days before finally being stored at –80°C until analysis. The frozen serum samples were thawed at room temperature for approximately one hour. Aliquots of 120 µl were carefully mixed with equal amounts of phosphate buffer in Eppendorf tubes, and transferred to 3 mm SampleJet tubes by syringe. A fill height of 4 cm was used amounting to approximately 180 µl.

^{1}H NMR protocol

Serum spectra were recorded at 310 K, using a one-dimensional NOESY (noesygppr1d) pulse sequence. A total of 32 scans were acquired, using 96k data points and 30 ppm spectral width. The spectra were processed with 0.3 Hz line broadening, automatically phase-corrected and aligned to the lactate signal at 1.32 ppm. Spectra were normalised to an ERETIC signal, functioning as an external reference. Details of the ^{1}H NMR protocol have been described previously.[1]

References

1 Jones PR, Rajalahti T, Resaland GK, *et al.* Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis* 2019; **288**: 186–93.

Table 1. Mean and standard deviation (SD) for each lipoprotein measure in absolute concentration units

Lipoprotein variable	Baseline	Follow-up
	Mean (SD)	Mean (SD)
CM PN (nmol·L^{-1})	0.240 (0.392)	0.220 (0.309)
VLDL L1 PN (nmol·L^{-1})	1.024 (1.252)	0.947 (1.028)
VLDL L2 PN (nmol·L^{-1})	4.315 (4.585)	3.978 (3.840)
VLDL L3 PN (nmol·L^{-1})	19.189 (10.628)	18.358 (9.813)
VLDL M PN (nmol·L^{-1})	29.151 (13.484)	28.751 (12.626)
VLDL S PN (nmol·L^{-1})	45.085 (11.105)	45.786 (11.055)
LDL L PN (nmol·L^{-1})	213.391 (44.846)	216.990 (43.153)
LDL M PN (nmol·L^{-1})	469.633 (101.065)	472.169 (97.716)
LDL S PN (nmol·L^{-1})	222.782 (49.953)	221.408 (48.913)
LDL VS PN (nmol·1^{-1})	0.176 (0.036)	0.176 (0.035)
HDL VL PN (nmol·L^{-1})	0.087 (0.035)	0.090 (0.036)
HDL L PN (nmol·L^{-1})	1639.967 (736.695)	1724.870 (757.571)
HDL M PN (nmol·L^{-1})	4327.068 (606.213)	4343.645 (587.757)
HDL S PN (nmol·L^{-1})	5233.284 (543.149)	5162.860 (569.243)
HDL VS PN (nmol·L^{-1})	2674.001 (249.148)	2635.574 (254.906)
CM C (mmol·L^{-1})	0.011 (0.014)	0.010 (0.011)
VLDL C (mmol·L^{-1})	0.642 (0.256)	0.641 (0.238)
VLDL L1 C (mmol·L^{-1})	0.020 (0.018)	0.019 (0.015)
VLDL L2 C (mmol·L^{-1})	0.041 (0.042)	0.040 (0.036)
VLDL L3 C (mmol·L^{-1})	0.205 (0.088)	0.200 (0.086)
VLDL M C (mmol·L^{-1})	0.164 (0.079)	0.163 (0.072)
VLDL S C (mmol·L^{-1})	0.208 (0.053)	0.213 (0.053)
LDL C (mmol·L^{-1})	2.234 (0.496)	2.254 (0.479)
LDL L C (mmol·L^{-1})	0.716 (0.165)	0.731 (0.157)
LDL M C (mmol·L^{-1})	0.968 (0.223)	0.974 (0.218)
LDL S C (mmol·L^{-1})	0.389 (0.093)	0.388 (0.091)
LDL VS C (mmol·L^{-1})	0.165 (0.035)	0.165 (0.034)
HDL C (mmol·L^{-1})	1.465 (0.262)	1.487 (0.257)
HDL VL C (mmol·L^{-1})	0.080 (0.033)	0.084 (0.035)
HDL L C (mmol·L^{-1})	0.332 (0.156)	0.350 (0.160)
HDL M C (mmol·L^{-1})	0.528 (0.085)	0.532 (0.081)
HDL S C (mmol·L^{-1})	0.377 (0.037)	0.373 (0.039)
HDL VS C (mmol·L^{-1})	0.148 (0.012)	0.147 (0.012)
Total C (mmol·L^{-1})	4.359 (0.673)	4.397 (0.645)
Non-HDL C (mmol·L^{-1})	2.894 (0.663)	2.910 (0.647)
CM TG (mmol·L^{-1})	0.026 (0.044)	0.024 (0.035)
VLDL TG (mmol·L^{-1})	0.432 (0.318)	0.407 (0.278)
VLDL L1 TG (mmol·L^{-1})	0.039 (0.053)	0.036 (0.043)
VLDL L2 TG (mmol·L^{-1})	0.096 (0.102)	0.088 (0.085)
VLDL L3 TG (mmol·L^{-1})	0.155 (0.113)	0.145 (0.101)
VLDL M TG (mmol·L^{-1})	0.096 (0.049)	0.092 (0.047)
VLDL S TG (mmol·L^{-1})	0.046 (0.014)	0.046 (0.013)
LDL TG (mmol·L^{-1})	0.192 (0.035)	0.191 (0.032)
LDL L TG (mmol·L^{-1})	0.076 (0.017)	0.076 (0.016)
LDL M TG (mmol·L^{-1})	0.076 (0.014)	0.076 (0.013)
LDL S TG (mmol·L^{-1})	0.029 (0.006)	0.029 (0.005)
LDL VS TG (mmol·L^{-1})	0.011 (0.003)	0.011 (0.003)

HDL TG (mmol·L^{-1})	0.104 (0.038)	0.104 (0.034)
HDL VL TG (mmol·L^{-1})	0.006 (0.003)	0.006 (0.002)
HDL L TG (mmol·L^{-1})	0.026 (0.010)	0.026 (0.010)
HDL M TG (mmol·L^{-1})	0.038 (0.016)	0.038 (0.014)
HDL S TG (mmol·L^{-1})	0.022 (0.010)	0.022 (0.009)
HDL VS TG (mmol·L^{-1})	0.011 (0.003)	0.010 (0.003)
Total TG (mmol·L^{-1})	0.748 (0.412)	0.723 (0.350)
VLDL size (nm)	42.560 (3.116)	42.231 (2.595)
LDL size (nm)	25.766 (0.135)	25.786 (0.134)
HDL size (nm)	10.873 (0.212)	10.900 (0.218)

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

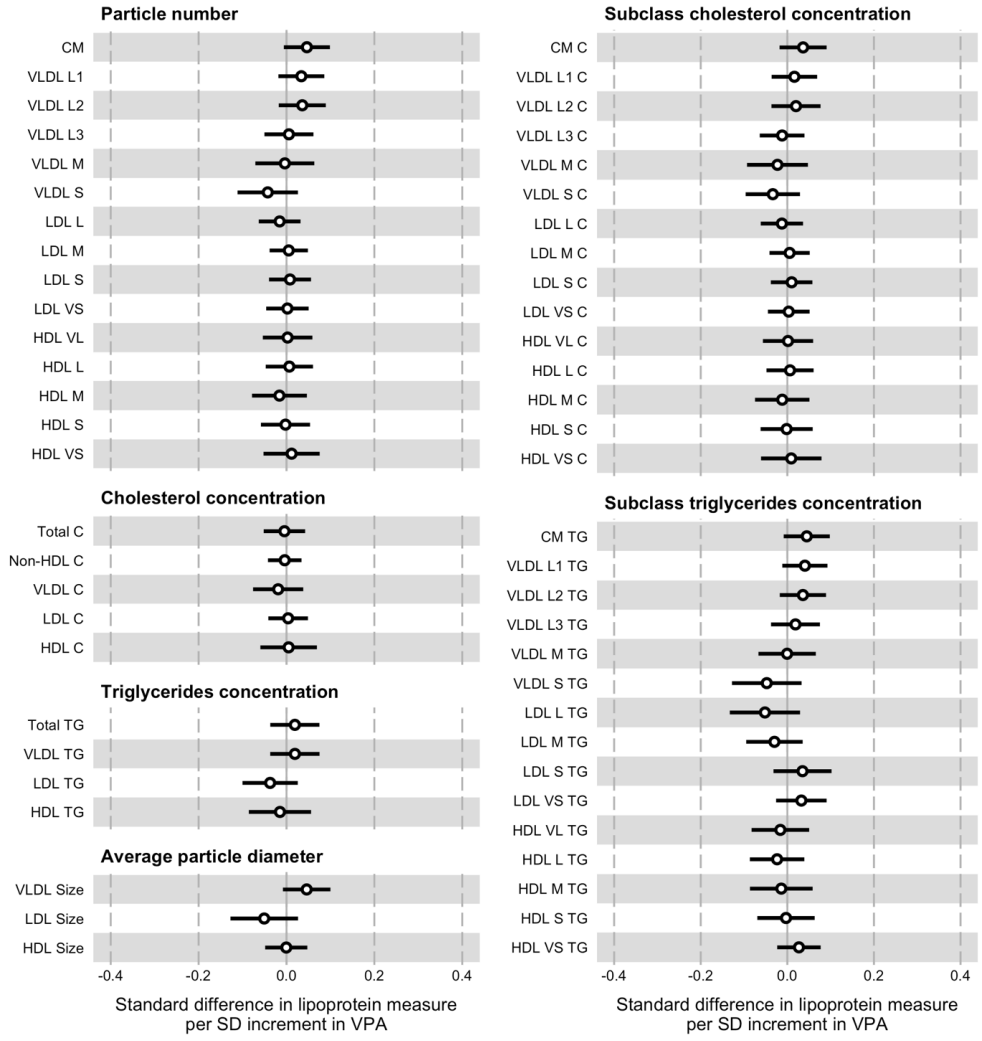
Table 2. Comparison of characteristics between those children included or not included in the analytical sample

Characteristic	Included		Excluded		Difference
	n (%)	Mean (SD)	*n* (%)	Mean (SD)	*p* value[a]
Baseline	762		367		
Age (years)		10.0 (0.3)	315	10.0 (0.4)	0.032
Sex					
Girls	378 (49.6)		163 (44.4)		0.102
Boys	384 (50.4)		204 (55.6)		
Anthropometry					
Height (m)		143.1 (6.7)	334	141.8 (6.9)	0.004
Weight (kg)		37.2 (8.1)	333	36.6 (7.9)	0.201
BMI ($kg \cdot m^{-2}$)		18.1 (3.0)	333	18 (2.9)	0.931
≥25	174 (22.8)		61 (18.3)		0.016
≥30	38 (5.0)		7 (2.1)		
Waist circumference (cm)		62.1 (7.6)	332	61.8 (7.3)	0.523
Parents' education[b]					
Upper secondary school	241 (31.6)		108 (35.2)		0.263
<4 years college/university	229 (30.1)		91 (29.6)		
≥4 years college/university	292 (38.3)		108 (35.2)		
Tanner stage[c]					
Stage 1	417 (54.7)		164 (51.4)		0.319
Stage 2	297 (39.0)		130 (40.8)		
Stage ≥3	48 (6.3)		25 (7.8)		
Physical activity					
VPA ($min \cdot d^{-1}$)		32.2 (16.2)	298	27.4 (14.9)	<0.001
MPA ($min \cdot d^{-1}$)		45.1 (12.8)	298	41.8 (14.1)	<0.001
LPA ($min \cdot d^{-1}$)		235.2 (36.7)	298	226.3 (42.5)	0.001
SED ($min \cdot d^{-1}$)		466.5 (57.5)	298	467 (65.2)	0.909
MVPA ≥60 ($min \cdot d^{-1}$)	563 (73.9)		189 (51.5)		<0.001
Lipid profile					

TC (mmol·L^{-1})		4.5 (0.7)	246	4.4 (0.7)	0.390
LDL-C (mmol·L^{-1})		2.5 (0.6)	246	2.5 (0.7)	0.945
HDL-C (mmol·L^{-1})		1.6 (0.3)	246	1.6 (0.3)	0.116
TG (mmol·L^{-1})[d]		0.7 [0.5, 0.9]	246	0.7 [0.5, 0.9]	0.931[e]
Follow-up	720		409		
Physical activity					
VPA (min·d^{-1})		27.6 (13.4)	387	24.3 (13.3)	<0.001
MPA (min·d^{-1})		40.3 (11.9)	387	40.1 (13.2)	0.831
LPA (min·d^{-1})		220.8 (34.8)	387	218.3 (39.0)	0.276
SED (min·d^{-1})		494.3 (52.1)	387	488.6 (65.5)	0.114
MVPA ≥60 (min·d^{-1})	451 (62.6)		212 (51.8)		<0.001
Lipid profile					
TC (mmol·L^{-1})		4.5 (0.6)	221	4.6 (0.7)	0.649
LDL-C (mmol·L^{-1})		2.6 (0.6)	221	2.6 (0.5)	0.641
HDL-C (mmol·L^{-1})		1.6 (0.3)	221	1.6 (0.3)	0.540
TG (mmol·L^{-1})[d]		0.6 [0.5, 0.9]	221	0.7 [0.5, 0.9]	0.224[e]

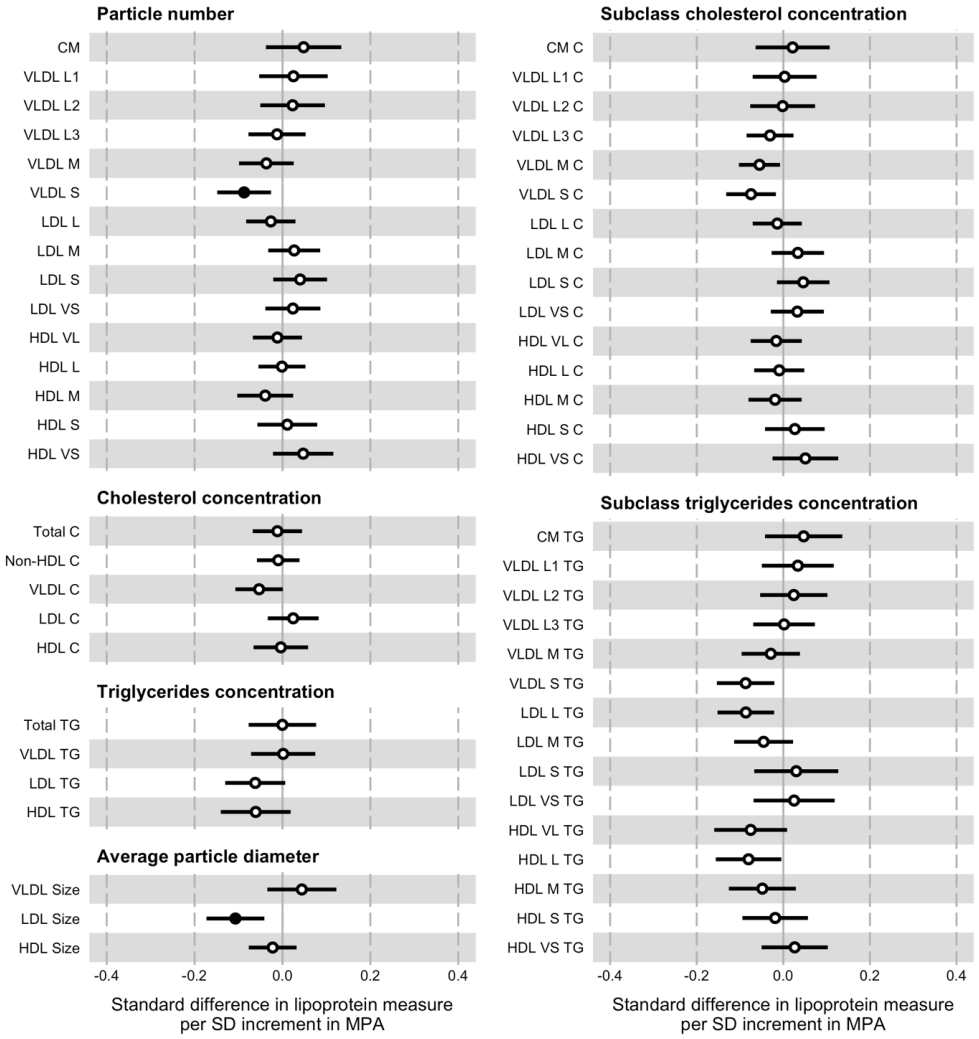
[a]Continuous characteristics compared using linear regression, categorical characteristics using binomial logistic regression.
[b]For comparison, dichotomised to 'Upper secondary school' and 'Higher than upper secondary school'.
[c]For comparison, dichotomised to Tanner stage 1 and Tanner stage ≥2.
[d]Median [IQR].
[e]Comparison performed using *log*(TG).
Abbreviations: BMI = body mass index; HDL-C = high-density lipoprotein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; LPA = light-intensity physical activity; MPA = moderate-intensity physical activity; SD = standard deviation; SED = sedentary time; VPA = vigorous-intensity physical activity; TC = total cholesterol; TG = triglycerides.

Figure 1. Associations between change in vigorous-intensity physical activity (VPA) and follow-up lipoprotein measures



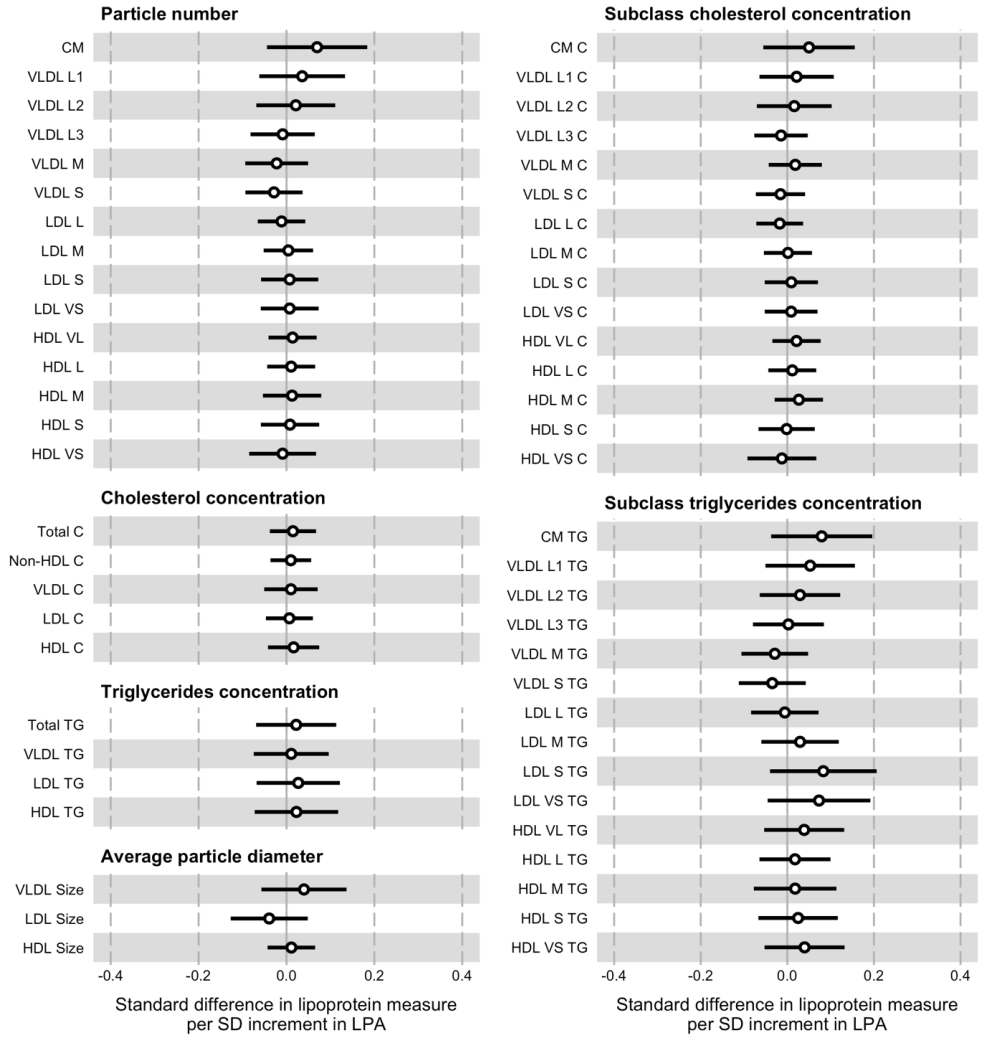
The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 2. Associations between change in moderate-intensity physical activity (MPA) and follow-up lipoprotein measures



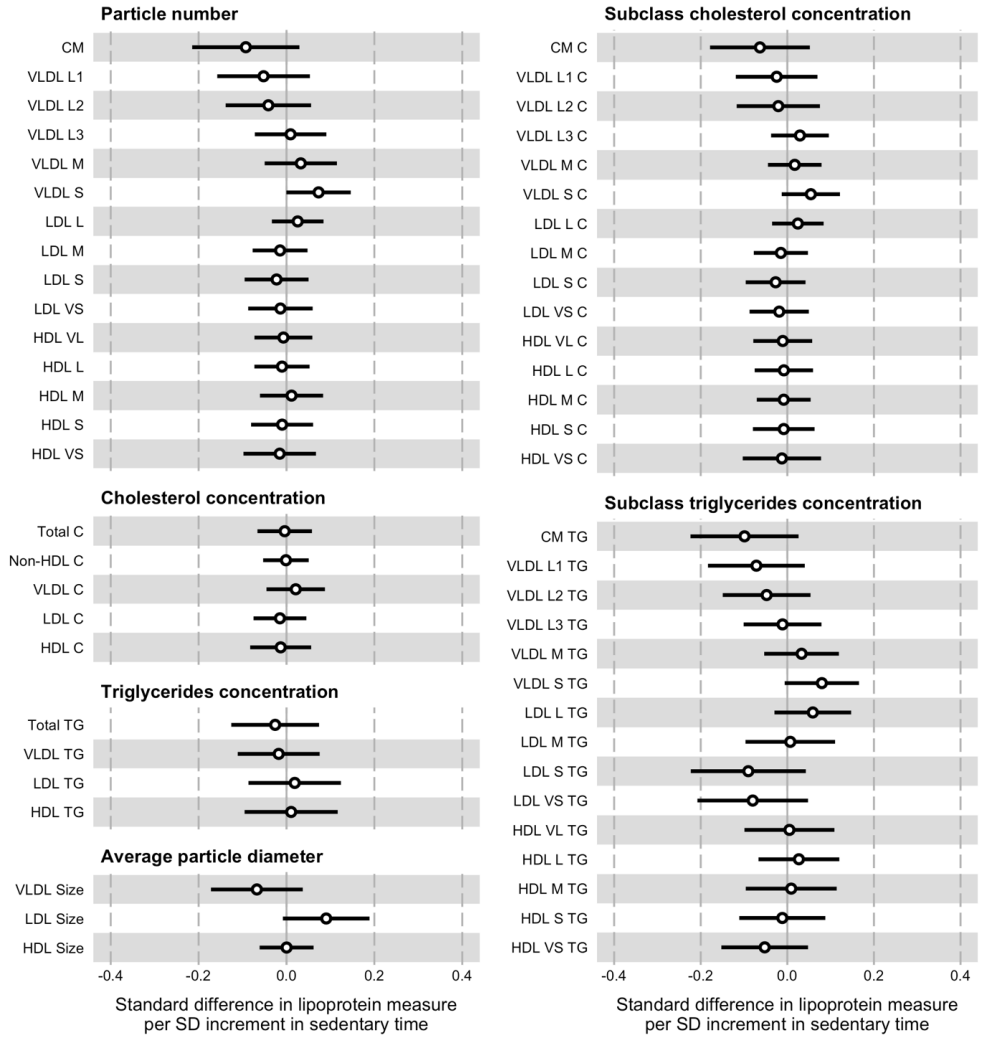
The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 3. Associations between change in light-intensity physical activity (LPA) and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Figure 4. Associations between change in sedentary time and follow-up lipoprotein measures



The association magnitudes are the standardised unit difference in lipoprotein measure per SD unit increment of change in activity variable (follow-up minus baseline). The models are adjusted for change in accelerometer wear time and baseline values of age, lipoprotein measure, parents' education, sex, sexual maturity, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable. Filled circles are p <0.01. Error bars are 95% confidence intervals.
Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 3. Associations between baseline vigorous-intensity physical activity (VPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0439	–0.0652	–0.0227	1.17E–04	–0.0304	–0.0468	–0.0140	4.71E–04
VLDL L1 PN (nmol·L^{-1})	–0.1318	–0.1983	–0.0653	2.06E–04	–0.0860	–0.1411	–0.0309	2.79E–03
VLDL L2 PN (nmol·L^{-1})	–0.4809	–0.7212	–0.2406	1.82E–04	–0.3136	–0.5226	–0.1047	3.95E–03
VLDL L3 PN (nmol·L^{-1})	–0.9019	–1.3944	–0.4095	5.44E–04	–0.5655	–1.0230	–0.1080	1.63E–02
VLDL M PN (nmol·L^{-1})	–0.8315	–1.5453	–0.1176	2.32E–02	–0.4367	–1.2215	0.3481	2.70E–01
VLDL S PN (nmol·L^{-1})	–0.1670	–0.7663	0.4322	5.79E–01	–0.0384	–0.6950	0.6183	9.07E–01
LDL L PN (nmol·L^{-1})	–1.1285	–3.0452	0.7881	2.43E–01	–0.9264	–2.9731	1.1202	3.68E–01
LDL M PN (nmol·L^{-1})	–4.5116	–9.7008	0.6776	8.71E–02	–3.8345	–9.1844	1.5155	1.57E–01
LDL S PN (nmol·L^{-1})	–2.8130	–5.8236	0.1976	6.65E–02	–2.3074	–5.2987	0.6840	1.28E–01
LDL VS PN (nmol·1^{-1})	–1.3579	–2.8924	0.1767	8.17E–02	–1.1370	–2.6677	0.3937	1.42E–01
HDL VL PN (nmol·L^{-1})	2.8404	–2.6701	8.3509	3.06E–01	1.7695	–3.6925	7.2314	5.19E–01
HDL L PN (nmol·L^{-1})	25.3609	–14.5915	65.3133	2.09E–01	17.7252	–22.0069	57.4572	3.75E–01
HDL M PN (nmol·L^{-1})	18.1486	–17.2347	53.5319	3.09E–01	8.6224	–25.6787	42.9235	6.17E–01
HDL S PN (nmol·L^{-1})	–18.4718	–50.9702	14.0266	2.60E–01	–10.4427	–42.6659	21.7806	5.19E–01
HDL VS PN (nmol·L^{-1})	–11.1428	–25.5859	3.3003	1.28E–01	–7.2357	–21.2975	6.8261	3.07E–01
CM C (mmol·L^{-1})	–0.0015	–0.0023	–0.0008	8.12E–05	–0.0010	–0.0016	–0.0005	7.39E–04
VLDL C (mmol·L^{-1})	–0.0148	–0.0243	–0.0052	3.01E–03	–0.0102	–0.0202	–0.0002	4.59E–02
VLDL L1 C (mmol·L^{-1})	–0.0018	–0.0027	–0.0009	1.23E–04	–0.0012	–0.0019	–0.0005	1.79E–03
VLDL L2 C (mmol·L^{-1})	–0.0041	–0.0062	–0.0020	2.57E–04	–0.0026	–0.0045	–0.0007	8.22E–03
VLDL L3 C (mmol·L^{-1})	–0.0058	–0.0094	–0.0021	2.58E–03	–0.0040	–0.0074	–0.0007	1.98E–02
VLDL M C (mmol·L^{-1})	–0.0048	–0.0086	–0.0011	1.27E–02	–0.0034	–0.0074	0.0005	8.63E–02
VLDL S C (mmol·L^{-1})	–0.0008	–0.0033	0.0018	5.43E–01	–0.0005	–0.0033	0.0023	7.39E–01
LDL C (mmol·L^{-1})	–0.0220	–0.0473	0.0033	8.65E–02	–0.0189	–0.0449	0.0072	1.53E–01
LDL L C (mmol·L^{-1})	–0.0030	–0.0101	0.0042	4.11E–01	–0.0024	–0.0100	0.0052	5.25E–01
LDL M C (mmol·L^{-1})	–0.0092	–0.0206	0.0023	1.16E–01	–0.0076	–0.0195	0.0042	2.01E–01
LDL S C (mmol·L^{-1})	–0.0054	–0.0109	0.0001	5.58E–02	–0.0044	–0.0099	0.0011	1.15E–01
LDL VS C (mmol·L^{-1})	–0.0020	–0.0042	0.0001	6.63E–02	–0.0017	–0.0039	0.0005	1.28E–01
HDL C (mmol·L^{-1})	0.0082	–0.0079	0.0242	3.12E–01	0.0041	–0.0116	0.0198	6.02E–01
HDL VL C (mmol·L^{-1})	0.0010	–0.0011	0.0030	3.53E–01	0.0005	–0.0015	0.0025	6.22E–01
HDL L C (mmol·L^{-1})	0.0053	–0.0032	0.0138	2.13E–01	0.0035	–0.0049	0.0119	4.07E–01
HDL M C (mmol·L^{-1})	0.0026	–0.0023	0.0076	2.90E–01	0.0011	–0.0038	0.0060	6.65E–01
HDL S C (mmol·L^{-1})	–0.0009	–0.0030	0.0013	4.34E–01	–0.0004	–0.0027	0.0018	6.94E–01

HDL VS C (mmol·L^{-1})	–0.0005	–0.0012	0.0003	2.48E–01	–0.0003	–0.0011	0.0005	4.48E–01
Total C (mmol·L^{-1})	–0.0242	–0.0567	0.0083	1.42E–01	–0.0231	–0.0566	0.0105	1.74E–01
Non-HDL C (mmol·L^{-1})	–0.0313	–0.0575	–0.0050	2.05E–02	–0.0271	–0.0528	–0.0014	3.93E–02
CM TG (mmol·L^{-1})	–0.0051	–0.0075	–0.0027	8.23E–05	–0.0036	–0.0054	–0.0018	2.30E–04
VLDL TG (mmol·L^{-1})	–0.0309	–0.0479	–0.0139	5.93E–04	–0.0193	–0.0348	–0.0037	1.60E–02
VLDL L1 TG (mmol·L^{-1})	–0.0060	–0.0089	–0.0032	8.48E–05	–0.0041	–0.0064	–0.0018	6.38E–04
VLDL L2 TG (mmol·L^{-1})	–0.0111	–0.0166	–0.0056	1.51E–04	–0.0074	–0.0120	–0.0027	2.42E–03
VLDL L3 TG (mmol·L^{-1})	–0.0110	–0.0170	–0.0050	5.56E–04	–0.0068	–0.0124	–0.0013	1.66E–02
VLDL M TG (mmol·L^{-1})	–0.0036	–0.0064	–0.0009	1.12E–02	–0.0019	–0.0049	0.0011	2.05E–01
VLDL S TG (mmol·L^{-1})	–0.0004	–0.0014	0.0006	4.46E–01	–0.0001	–0.0011	0.0010	9.11E–01
LDL TG (mmol·L^{-1})	–0.0012	–0.0031	0.0008	2.44E–01	–0.0009	–0.0028	0.0011	3.78E–01
LDL L TG (mmol·L^{-1})	–0.0003	–0.0016	0.0009	5.95E–01	–0.0001	–0.0014	0.0011	8.26E–01
LDL M TG (mmol·L^{-1})	–0.0003	–0.0012	0.0005	4.26E–01	–0.0003	–0.0011	0.0005	4.83E–01
LDL S TG (mmol·L^{-1})	–0.0007	–0.0010	–0.0003	4.40E–04	–0.0005	–0.0008	–0.0002	8.12E–04
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0002	4.51E–05	–0.0003	–0.0004	–0.0002	4.20E–05
HDL TG (mmol·L^{-1})	–0.0014	–0.0037	0.0009	2.33E–01	–0.0008	–0.0032	0.0017	5.43E–01
HDL VL TG (mmol·L^{-1})	0.0000	–0.0001	0.0001	8.32E–01	0.0000	–0.0002	0.0001	5.00E–01
HDL L TG (mmol·L^{-1})	0.0004	–0.0003	0.0010	2.40E–01	0.0002	–0.0004	0.0009	5.24E–01
HDL M TG (mmol·L^{-1})	–0.0007	–0.0018	0.0004	1.93E–01	–0.0003	–0.0015	0.0008	5.82E–01
HDL S TG (mmol·L^{-1})	–0.0007	–0.0014	0.0000	4.77E–02	–0.0004	–0.0011	0.0003	3.04E–01
HDL VS TG (mmol·L^{-1})	–0.0003	–0.0005	–0.0002	1.14E–04	–0.0002	–0.0004	–0.0001	1.24E–03
Total TG (mmol·L^{-1})	–0.0399	–0.0613	–0.0186	4.34E–04	–0.0254	–0.0447	–0.0061	1.07E–02
VLDL size (nm)	–0.3647	–0.5437	–0.1856	1.44E–04	–0.2539	–0.4038	–0.1041	1.27E–03
LDL size (nm)	0.0104	–0.0007	0.0215	6.59E–02	0.0084	–0.0028	0.0196	1.38E–01
HDL size (nm)	0.0077	–0.0029	0.0183	1.52E–01	0.0051	–0.0056	0.0157	3.45E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 4. Associations between baseline moderate-intensity physical activity (MPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0382	–0.0642	–0.0122	4.78E–03	–0.0301	–0.0548	–0.0055	1.75E–02
VLDL L1 PN (nmol·L^{-1})	–0.1139	–0.1973	–0.0306	8.29E–03	–0.0876	–0.1657	–0.0096	2.84E–02
VLDL L2 PN (nmol·L^{-1})	–0.4173	–0.7279	–0.1067	9.37E–03	–0.3211	–0.6129	–0.0294	3.16E–02
VLDL L3 PN (nmol·L^{-1})	–0.7346	–1.4111	–0.0581	3.38E–02	–0.5477	–1.1816	0.0862	8.90E–02
VLDL M PN (nmol·L^{-1})	–0.5595	–1.3445	0.2255	1.59E–01	–0.3334	–1.0787	0.4120	3.74E–01
VLDL S PN (nmol·L^{-1})	0.1848	–0.4951	0.8647	5.88E–01	0.2700	–0.4295	0.9695	4.43E–01
LDL L PN (nmol·L^{-1})	0.3586	–2.3233	3.0405	7.90E–01	0.5238	–2.2372	3.2847	7.05E–01
LDL M PN (nmol·L^{-1})	–1.9253	–8.2246	4.3741	5.43E–01	–1.4106	–7.9024	5.0812	6.65E–01
LDL S PN (nmol·L^{-1})	–2.0133	–5.3129	1.2863	2.27E–01	–1.6625	–5.0433	1.7183	3.29E–01
LDL VS PN (nmol·1^{-1})	–1.0403	–2.6494	0.5689	2.01E–01	–0.8857	–2.5288	0.7574	2.85E–01
HDL VL PN (nmol·L^{-1})	–1.0764	–6.0858	3.9331	6.69E–01	–1.7402	–6.8905	3.4102	5.01E–01
HDL L PN (nmol·L^{-1})	–6.4509	–46.3380	33.4362	7.47E–01	–10.9988	–52.0761	30.0785	5.94E–01
HDL M PN (nmol·L^{-1})	9.9821	–34.2439	54.2081	6.53E–01	4.3478	–40.5196	49.2152	8.47E–01
HDL S PN (nmol·L^{-1})	–1.0307	–42.7645	40.7031	9.61E–01	4.5043	–38.3276	47.3362	8.34E–01
HDL VS PN (nmol·L^{-1})	–8.3757	–28.0431	11.2917	3.97E–01	–5.7361	–26.3380	14.8658	5.79E–01
CM C (mmol·L^{-1})	–0.0014	–0.0023	–0.0005	4.35E–03	–0.0011	–0.0020	–0.0002	1.50E–02
VLDL C (mmol·L^{-1})	–0.0078	–0.0193	0.0037	1.81E–01	–0.0052	–0.0162	0.0059	3.55E–01
VLDL L1 C (mmol·L^{-1})	–0.0015	–0.0026	–0.0004	9.61E–03	–0.0012	–0.0022	–0.0001	2.90E–02
VLDL L2 C (mmol·L^{-1})	–0.0035	–0.0062	–0.0008	1.26E–02	–0.0026	–0.0052	–0.0001	3.98E–02
VLDL L3 C (mmol·L^{-1})	–0.0047	–0.0098	0.0003	6.53E–02	–0.0037	–0.0086	0.0011	1.25E–01
VLDL M C (mmol·L^{-1})	–0.0023	–0.0056	0.0010	1.65E–01	–0.0015	–0.0047	0.0017	3.53E–01
VLDL S C (mmol·L^{-1})	0.0008	–0.0022	0.0038	5.90E–01	0.0010	–0.0021	0.0042	5.12E–01
LDL C (mmol·L^{-1})	–0.0120	–0.0427	0.0187	4.38E–01	–0.0096	–0.0412	0.0220	5.46E–01
LDL L C (mmol·L^{-1})	0.0007	–0.0087	0.0100	8.90E–01	0.0011	–0.0087	0.0108	8.24E–01
LDL M C (mmol·L^{-1})	–0.0049	–0.0191	0.0094	4.94E–01	–0.0038	–0.0185	0.0109	6.08E–01
LDL S C (mmol·L^{-1})	–0.0040	–0.0101	0.0022	2.00E–01	–0.0033	–0.0096	0.0030	3.00E–01
LDL VS C (mmol·L^{-1})	–0.0015	–0.0039	0.0008	1.87E–01	–0.0013	–0.0037	0.0011	2.76E–01
HDL C (mmol·L^{-1})	–0.0012	–0.0177	0.0152	8.81E–01	–0.0034	–0.0199	0.0130	6.78E–01
HDL VL C (mmol·L^{-1})	–0.0006	–0.0025	0.0014	5.63E–01	–0.0008	–0.0028	0.0011	4.02E–01
HDL L C (mmol·L^{-1})	–0.0013	–0.0098	0.0071	7.56E–01	–0.0024	–0.0111	0.0063	5.86E–01
HDL M C (mmol·L^{-1})	0.0005	–0.0055	0.0065	8.72E–01	–0.0004	–0.0064	0.0056	8.92E–01
HDL S C (mmol·L^{-1})	0.0002	–0.0027	0.0031	8.84E–01	0.0005	–0.0025	0.0035	7.41E–01

HDL VS C (mmol·L^{-1})	–0.0003	–0.0014	0.0007	5.17E–01	–0.0002	–0.0013	0.0008	6.70E–01
Total C (mmol·L^{-1})	–0.0158	–0.0577	0.0260	4.51E–01	–0.0147	–0.0572	0.0277	4.90E–01
Non-HDL C (mmol·L^{-1})	–0.0140	–0.0498	0.0217	4.35E–01	–0.0110	–0.0464	0.0245	5.38E–01
CM TG (mmol·L^{-1})	–0.0045	–0.0074	–0.0015	3.57E–03	–0.0036	–0.0064	–0.0008	1.34E–02
VLDL TG (mmol·L^{-1})	–0.0258	–0.0477	–0.0040	2.15E–02	–0.0192	–0.0397	0.0014	6.70E–02
VLDL L1 TG (mmol·L^{-1})	–0.0053	–0.0089	–0.0017	4.61E–03	–0.0042	–0.0075	–0.0008	1.68E–02
VLDL L2 TG (mmol·L^{-1})	–0.0096	–0.0167	–0.0025	8.82E–03	–0.0074	–0.0141	–0.0007	3.00E–02
VLDL L3 TG (mmol·L^{-1})	–0.0092	–0.0171	–0.0012	2.41E–02	–0.0068	–0.0142	0.0006	7.25E–02
VLDL M TG (mmol·L^{-1})	–0.0027	–0.0061	0.0006	1.07E–01	–0.0017	–0.0049	0.0015	2.81E–01
VLDL S TG (mmol·L^{-1})	–0.0001	–0.0010	0.0007	7.50E–01	0.0001	–0.0008	0.0009	8.94E–01
LDL TG (mmol·L^{-1})	–0.0005	–0.0027	0.0016	6.26E–01	–0.0003	–0.0025	0.0018	7.58E–01
LDL L TG (mmol·L^{-1})	0.0000	–0.0012	0.0012	9.75E–01	0.0001	–0.0010	0.0013	8.02E–01
LDL M TG (mmol·L^{-1})	–0.0001	–0.0011	0.0009	8.76E–01	0.0000	–0.0011	0.0010	9.32E–01
LDL S TG (mmol·L^{-1})	–0.0007	–0.0011	–0.0003	1.32E–03	–0.0006	–0.0010	–0.0002	3.01E–03
LDL VS TG (mmol·L^{-1})	–0.0004	–0.0006	–0.0001	1.56E–03	–0.0003	–0.0005	–0.0001	4.76E–03
HDL TG (mmol·L^{-1})	–0.0013	–0.0035	0.0010	2.73E–01	–0.0009	–0.0032	0.0014	4.59E–01
HDL VL TG (mmol·L^{-1})	–0.0001	–0.0002	0.0001	3.22E–01	–0.0001	–0.0003	0.0001	2.29E–01
HDL L TG (mmol·L^{-1})	0.0002	–0.0005	0.0008	6.25E–01	0.0000	–0.0006	0.0007	9.04E–01
HDL M TG (mmol·L^{-1})	–0.0004	–0.0014	0.0005	3.67E–01	–0.0002	–0.0012	0.0008	6.82E–01
HDL S TG (mmol·L^{-1})	–0.0004	–0.0011	0.0002	1.74E–01	–0.0002	–0.0009	0.0004	4.39E–01
HDL VS TG (mmol·L^{-1})	–0.0003	–0.0005	–0.0001	8.11E–03	–0.0002	–0.0004	0.0000	2.94E–02
Total TG (mmol·L^{-1})	–0.0337	–0.0610	–0.0064	1.66E–02	–0.0253	–0.0510	0.0005	5.42E–02
VLDL size (nm)	–0.3250	–0.5441	–0.1059	4.36E–03	–0.2601	–0.4685	–0.0516	1.54E–02
LDL size (nm)	0.0121	0.0016	0.0226	2.46E–02	0.0109	–0.0002	0.0219	5.40E–02
HDL size (nm)	–0.0019	–0.0131	0.0093	7.35E–01	–0.0035	–0.0152	0.0082	5.50E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 5. Associations between baseline light-intensity physical activity (LPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0185	–0.0431	0.0062	1.40E–01	–0.0172	–0.0415	0.0070	1.60E–01
VLDL L1 PN (nmol·L^{-1})	–0.0453	–0.1214	0.0309	2.39E–01	–0.0419	–0.1143	0.0305	2.51E–01
VLDL L2 PN (nmol·L^{-1})	–0.1762	–0.4437	0.0914	1.93E–01	–0.1652	–0.4170	0.0866	1.94E–01
VLDL L3 PN (nmol·L^{-1})	–0.0125	–0.5968	0.5717	9.66E–01	0.0079	–0.5278	0.5436	9.76E–01
VLDL M PN (nmol·L^{-1})	0.0810	–0.6827	0.8446	8.33E–01	0.1006	–0.6010	0.8022	7.75E–01
VLDL S PN (nmol·L^{-1})	0.5186	–0.3181	1.3553	2.20E–01	0.5320	–0.3045	1.3686	2.08E–01
LDL L PN (nmol·L^{-1})	2.5262	0.0932	4.9591	4.21E–02	2.5597	0.1172	5.0022	4.03E–02
LDL M PN (nmol·L^{-1})	5.4295	0.1334	10.7256	4.47E–02	5.5798	0.3067	10.8529	3.85E–02
LDL S PN (nmol·L^{-1})	2.4162	–0.5966	5.4289	1.14E–01	2.5299	–0.4694	5.5293	9.66E–02
LDL VS PN (nmol·1^{-1})	0.7679	–0.6816	2.2175	2.93E–01	0.8123	–0.6372	2.2618	2.66E–01
HDL VL PN (nmol·L^{-1})	–7.5808	–12.0786	–3.0830	1.34E–03	–7.7217	–12.0542	–3.3893	7.41E–04
HDL L PN (nmol·L^{-1})	–50.7116	–83.0841	–18.3390	2.71E–03	–51.6776	–82.9954	–20.3598	1.66E–03
HDL M PN (nmol·L^{-1})	–22.6285	–58.4923	13.2353	2.11E–01	–23.6760	–58.9766	11.6246	1.85E–01
HDL S PN (nmol·L^{-1})	34.9046	–3.9806	73.7898	7.75E–02	36.1746	–2.2388	74.5881	6.44E–02
HDL VS PN (nmol·L^{-1})	16.0605	–1.1641	33.2851	6.70E–02	16.7175	–0.4156	33.8506	5.56E–02
CM C (mmol·L^{-1})	–0.0007	–0.0015	0.0002	1.44E–01	–0.0006	–0.0015	0.0002	1.55E–01
VLDL C (mmol·L^{-1})	0.0016	–0.0118	0.0150	8.10E–01	0.0020	–0.0109	0.0149	7.59E–01
VLDL L1 C (mmol·L^{-1})	–0.0003	–0.0013	0.0007	5.59E–01	–0.0003	–0.0012	0.0007	5.85E–01
VLDL L2 C (mmol·L^{-1})	–0.0010	–0.0034	0.0014	4.06E–01	–0.0009	–0.0032	0.0013	4.13E–01
VLDL L3 C (mmol·L^{-1})	0.0018	–0.0023	0.0059	3.92E–01	0.0019	–0.0020	0.0058	3.29E–01
VLDL M C (mmol·L^{-1})	–0.0011	–0.0049	0.0028	5.84E–01	–0.0010	–0.0047	0.0027	5.91E–01
VLDL S C (mmol·L^{-1})	0.0017	–0.0021	0.0054	3.72E–01	0.0017	–0.0021	0.0055	3.66E–01
LDL C (mmol·L^{-1})	0.0212	–0.0048	0.0471	1.08E–01	0.0218	–0.0041	0.0477	9.69E–02
LDL L C (mmol·L^{-1})	0.0085	0.0002	0.0168	4.58E–02	0.0086	0.0002	0.0170	4.55E–02
LDL M C (mmol·L^{-1})	0.0127	0.0009	0.0246	3.52E–02	0.0131	0.0013	0.0248	3.05E–02
LDL S C (mmol·L^{-1})	0.0041	–0.0015	0.0096	1.45E–01	0.0043	–0.0012	0.0098	1.24E–01
LDL VS C (mmol·L^{-1})	0.0012	–0.0009	0.0033	2.52E–01	0.0013	–0.0008	0.0034	2.27E–01
HDL C (mmol·L^{-1})	–0.0152	–0.0277	–0.0027	1.79E–02	–0.0155	–0.0274	–0.0036	1.19E–02
HDL VL C (mmol·L^{-1})	–0.0027	–0.0045	–0.0009	3.19E–03	–0.0028	–0.0045	–0.0011	1.85E–03
HDL L C (mmol·L^{-1})	–0.0109	–0.0185	–0.0034	5.22E–03	–0.0112	–0.0184	–0.0039	3.30E–03
HDL M C (mmol·L^{-1})	–0.0037	–0.0081	0.0008	1.04E–01	–0.0038	–0.0080	0.0004	7.61E–02
HDL S C (mmol·L^{-1})	0.0024	–0.0002	0.0051	7.32E–02	0.0025	–0.0002	0.0051	6.51E–02

HDL VS C (mmol·L^{-1})	0.0007	–0.0002	0.0016	1.06E–01	0.0008	–0.0001	0.0017	9.79E–02
Total C (mmol·L^{-1})	0.0096	–0.0258	0.0449	5.90E–01	0.0098	–0.0255	0.0452	5.79E–01
Non-HDL C (mmol·L^{-1})	0.0233	–0.0097	0.0562	1.63E–01	0.0241	–0.0085	0.0568	1.45E–01
CM TG (mmol·L^{-1})	–0.0020	–0.0048	0.0008	1.57E–01	–0.0019	–0.0046	0.0009	1.82E–01
VLDL TG (mmol·L^{-1})	–0.0052	–0.0243	0.0138	5.85E–01	–0.0045	–0.0223	0.0133	6.14E–01
VLDL L1 TG (mmol·L^{-1})	–0.0022	–0.0054	0.0010	1.69E–01	–0.0021	–0.0052	0.0010	1.84E–01
VLDL L2 TG (mmol·L^{-1})	–0.0034	–0.0095	0.0027	2.68E–01	–0.0031	–0.0089	0.0026	2.82E–01
VLDL L3 TG (mmol·L^{-1})	–0.0014	–0.0081	0.0053	6.85E–01	–0.0011	–0.0073	0.0051	7.20E–01
VLDL M TG (mmol·L^{-1})	0.0006	–0.0026	0.0037	7.23E–01	0.0007	–0.0023	0.0036	6.56E–01
VLDL S TG (mmol·L^{-1})	0.0007	–0.0004	0.0017	2.02E–01	0.0007	–0.0003	0.0017	1.77E–01
LDL TG (mmol·L^{-1})	0.0010	–0.0015	0.0035	4.36E–01	0.0010	–0.0015	0.0035	4.26E–01
LDL L TG (mmol·L^{-1})	0.0007	–0.0005	0.0019	2.65E–01	0.0007	–0.0005	0.0020	2.53E–01
LDL M TG (mmol·L^{-1})	0.0003	–0.0007	0.0014	5.48E–01	0.0003	–0.0007	0.0014	5.42E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0008	0.0000	5.07E–02	–0.0004	–0.0008	0.0000	5.89E–02
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	8.27E–02	–0.0002	–0.0004	0.0000	9.61E–02
HDL TG (mmol·L^{-1})	–0.0009	–0.0036	0.0018	5.09E–01	–0.0009	–0.0036	0.0019	5.30E–01
HDL VL TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	1.85E–02	–0.0002	–0.0004	0.0000	1.61E–02
HDL L TG (mmol·L^{-1})	–0.0003	–0.0010	0.0004	3.80E–01	–0.0003	–0.0010	0.0003	3.41E–01
HDL M TG (mmol·L^{-1})	0.0000	–0.0012	0.0012	9.99E–01	0.0000	–0.0011	0.0012	9.61E–01
HDL S TG (mmol·L^{-1})	0.0002	–0.0005	0.0009	6.41E–01	0.0002	–0.0005	0.0009	5.77E–01
HDL VS TG (mmol·L^{-1})	0.0000	–0.0002	0.0001	6.11E–01	0.0000	–0.0002	0.0001	6.56E–01
Total TG (mmol·L^{-1})	–0.0093	–0.0342	0.0156	4.57E–01	–0.0083	–0.0319	0.0152	4.81E–01
VLDL size (nm)	–0.1168	–0.2955	0.0620	1.96E–01	–0.1087	–0.2802	0.0627	2.09E–01
LDL size (nm)	0.0015	–0.0100	0.0130	7.94E–01	0.0013	–0.0103	0.0129	8.25E–01
HDL size (nm)	–0.0158	–0.0261	–0.0055	3.34E–03	–0.0162	–0.0261	–0.0064	1.72E–03

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 6. Associations between baseline sedentary time and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0435	0.0141	0.0728	4.45E–03	0.0346	0.0074	0.0617	1.35E–02
VLDL L1 PN (nmol·L^{-1})	0.1218	0.0304	0.2132	9.92E–03	0.0926	0.0097	0.1754	2.92E–02
VLDL L2 PN (nmol·L^{-1})	0.4531	0.1276	0.7787	7.23E–03	0.3476	0.0522	0.6430	2.19E–02
VLDL L3 PN (nmol·L^{-1})	0.5786	–0.1062	1.2634	9.61E–02	0.3684	–0.2470	0.9838	2.36E–01
VLDL M PN (nmol·L^{-1})	0.4283	–0.4507	1.3074	3.33E–01	0.1824	–0.6441	1.0088	6.60E–01
VLDL S PN (nmol·L^{-1})	–0.3936	–1.2882	0.5011	3.82E–01	–0.4876	–1.4312	0.4560	3.05E–01
LDL L PN (nmol·L^{-1})	–1.6423	–4.7158	1.4313	2.89E–01	–1.8430	–5.0281	1.3421	2.51E–01
LDL M PN (nmol·L^{-1})	–1.9206	–8.9266	5.0854	5.85E–01	–2.5777	–9.6757	4.5202	4.70E–01
LDL S PN (nmol·L^{-1})	–0.2138	–4.0090	3.5815	9.11E–01	–0.6610	–4.4907	3.1687	7.31E–01
LDL VS PN (nmol·1^{-1})	0.2335	–1.6192	2.0863	8.02E–01	0.0429	–1.8255	1.9114	9.63E–01
HDL VL PN (nmol·L^{-1})	5.2069	0.0091	10.4048	4.96E–02	6.0354	0.8816	11.1893	2.25E–02
HDL L PN (nmol·L^{-1})	32.2079	–6.2829	70.6988	9.93E–02	37.9489	–0.1736	76.0714	5.10E–02
HDL M PN (nmol·L^{-1})	8.5382	–35.2908	52.3673	6.98E–01	15.1323	–28.6523	58.9169	4.92E–01
HDL S PN (nmol·L^{-1})	–19.3194	–60.5987	21.9599	3.53E–01	–25.7449	–66.8657	15.3760	2.15E–01
HDL VS PN (nmol·L^{-1})	–5.6793	–25.8369	14.4783	5.75E–01	–8.8155	–29.5220	11.8910	3.97E–01
CM C (mmol·L^{-1})	0.0015	0.0005	0.0026	5.03E–03	0.0012	0.0003	0.0022	1.44E–02
VLDL C (mmol·L^{-1})	0.0068	–0.0076	0.0213	3.48E–01	0.0038	–0.0102	0.0178	5.87E–01
VLDL L1 C (mmol·L^{-1})	0.0014	0.0002	0.0026	2.46E–02	0.0010	–0.0001	0.0021	6.35E–02
VLDL L2 C (mmol·L^{-1})	0.0034	0.0005	0.0063	2.12E–02	0.0025	–0.0001	0.0051	5.91E–02
VLDL L3 C (mmol·L^{-1})	0.0022	–0.0029	0.0073	3.89E–01	0.0011	–0.0037	0.0058	6.56E–01
VLDL M C (mmol·L^{-1})	0.0034	–0.0007	0.0076	1.06E–01	0.0026	–0.0015	0.0067	2.13E–01
VLDL S C (mmol·L^{-1})	–0.0012	–0.0052	0.0027	5.35E–01	–0.0015	–0.0058	0.0028	4.90E–01
LDL C (mmol·L^{-1})	–0.0044	–0.0389	0.0300	7.98E–01	–0.0074	–0.0423	0.0275	6.72E–01
LDL L C (mmol·L^{-1})	–0.0057	–0.0160	0.0046	2.72E–01	–0.0062	–0.0170	0.0045	2.51E–01
LDL M C (mmol·L^{-1})	–0.0050	–0.0206	0.0107	5.28E–01	–0.0064	–0.0223	0.0095	4.24E–01
LDL S C (mmol·L^{-1})	0.0000	–0.0070	0.0071	9.89E–01	–0.0008	–0.0079	0.0063	8.22E–01
LDL VS C (mmol·L^{-1})	0.0003	–0.0024	0.0030	8.30E–01	0.0000	–0.0027	0.0027	9.99E–01
HDL C (mmol·L^{-1})	0.0094	–0.0066	0.0254	2.46E–01	0.0120	–0.0034	0.0275	1.25E–01
HDL VL C (mmol·L^{-1})	0.0019	–0.0001	0.0040	6.11E–02	0.0023	0.0003	0.0043	2.75E–02
HDL L C (mmol·L^{-1})	0.0070	–0.0017	0.0156	1.11E–01	0.0083	–0.0003	0.0169	5.71E–02
HDL M C (mmol·L^{-1})	0.0018	–0.0039	0.0075	5.28E–01	0.0029	–0.0027	0.0084	3.02E–01
HDL S C (mmol·L^{-1})	–0.0016	–0.0046	0.0014	2.82E–01	–0.0019	–0.0049	0.0010	1.98E–01

HDL VS C (mmol·L^{-1})	–0.0003	–0.0014	0.0008	5.95E–01	–0.0004	–0.0016	0.0007	4.71E–01
Total C (mmol·L^{-1})	0.0068	–0.0405	0.0542	7.74E–01	0.0054	–0.0425	0.0533	8.22E–01
Non-HDL C (mmol·L^{-1})	–0.0019	–0.0440	0.0402	9.28E–01	–0.0057	–0.0474	0.0359	7.84E–01
CM TG (mmol·L^{-1})	0.0049	0.0016	0.0083	4.21E–03	0.0039	0.0009	0.0070	1.29E–02
VLDL TG (mmol·L^{-1})	0.0240	0.0015	0.0464	3.70E–02	0.0166	–0.0038	0.0371	1.08E–01
VLDL L1 TG (mmol·L^{-1})	0.0057	0.0018	0.0096	4.81E–03	0.0045	0.0009	0.0080	1.46E–02
VLDL L2 TG (mmol·L^{-1})	0.0099	0.0025	0.0173	9.50E–03	0.0075	0.0008	0.0142	2.90E–02
VLDL L3 TG (mmol·L^{-1})	0.0081	0.0002	0.0160	4.51E–02	0.0055	–0.0017	0.0127	1.29E–01
VLDL M TG (mmol·L^{-1})	0.0018	–0.0017	0.0053	3.02E–01	0.0007	–0.0025	0.0040	6.60E–01
VLDL S TG (mmol·L^{-1})	–0.0003	–0.0014	0.0008	5.55E–01	–0.0005	–0.0017	0.0006	3.34E–01
LDL TG (mmol·L^{-1})	–0.0001	–0.0031	0.0028	9.24E–01	–0.0004	–0.0032	0.0025	8.08E–01
LDL L TG (mmol·L^{-1})	–0.0004	–0.0019	0.0011	5.87E–01	–0.0005	–0.0020	0.0009	4.65E–01
LDL M TG (mmol·L^{-1})	–0.0001	–0.0013	0.0012	8.95E–01	–0.0001	–0.0014	0.0011	8.46E–01
LDL S TG (mmol·L^{-1})	0.0008	0.0003	0.0012	1.45E–03	0.0007	0.0002	0.0011	2.75E–03
LDL VS TG (mmol·L^{-1})	0.0004	0.0002	0.0007	1.69E–03	0.0003	0.0001	0.0006	4.14E–03
HDL TG (mmol·L^{-1})	0.0017	–0.0012	0.0046	2.54E–01	0.0013	–0.0017	0.0043	4.02E–01
HDL VL TG (mmol·L^{-1})	0.0002	0.0000	0.0004	4.71E–02	0.0002	0.0000	0.0004	2.76E–02
HDL L TG (mmol·L^{-1})	0.0000	–0.0007	0.0008	9.25E–01	0.0002	–0.0006	0.0009	6.65E–01
HDL M TG (mmol·L^{-1})	0.0004	–0.0008	0.0017	4.96E–01	0.0002	–0.0011	0.0014	7.86E–01
HDL S TG (mmol·L^{-1})	0.0003	–0.0005	0.0011	4.68E–01	0.0001	–0.0007	0.0008	8.59E–01
HDL VS TG (mmol·L^{-1})	0.0002	0.0000	0.0005	2.14E–02	0.0002	0.0000	0.0004	6.22E–02
Total TG (mmol·L^{-1})	0.0332	0.0040	0.0624	2.68E–02	0.0240	–0.0029	0.0508	7.89E–02
VLDL size (nm)	0.3324	0.1090	0.5558	4.25E–03	0.2612	0.0569	0.4656	1.32E–02
LDL size (nm)	–0.0087	–0.0217	0.0043	1.84E–01	–0.0073	–0.0211	0.0065	2.92E–01
HDL size (nm)	0.0100	–0.0013	0.0214	8.24E–02	0.0121	0.0009	0.0233	3.53E–02

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of activity variable.
Model 1 is adjusted for baseline values of accelerometer wear time, age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 7. Associations between change in vigorous-intensity physical activity (VPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0108	–0.0042	0.0258	1.53E–01	0.0140	–0.0018	0.0299	8.21E–02
VLDL L1 PN (nmol·L^{-1})	0.0235	–0.0249	0.0719	3.35E–01	0.0345	–0.0189	0.0879	2.01E–01
VLDL L2 PN (nmol·L^{-1})	0.0966	–0.0874	0.2806	2.97E–01	0.1375	–0.0677	0.3428	1.85E–01
VLDL L3 PN (nmol·L^{-1})	–0.0185	–0.5147	0.4776	9.41E–01	0.0546	–0.4946	0.6039	8.43E–01
VLDL M PN (nmol·L^{-1})	–0.1108	–0.9120	0.6903	7.83E–01	–0.0480	–0.9019	0.8058	9.11E–01
VLDL S PN (nmol·L^{-1})	–0.4840	–1.2367	0.2687	2.03E–01	–0.4723	–1.2328	0.2883	2.19E–01
LDL L PN (nmol·L^{-1})	–0.7025	–2.7500	1.3449	4.95E–01	–0.6772	–2.7441	1.3896	5.14E–01
LDL M PN (nmol·L^{-1})	0.4207	–3.8552	4.6965	8.44E–01	0.5066	–3.8003	4.8135	8.15E–01
LDL S PN (nmol·L^{-1})	0.3272	–2.0146	2.6690	7.81E–01	0.3961	–1.9682	2.7604	7.38E–01
LDL VS PN (nmol·1^{-1})	0.0164	–1.1083	1.1411	9.77E–01	0.0496	–1.0897	1.1888	9.31E–01
HDL VL PN (nmol·L^{-1})	0.4299	–4.8948	5.7546	8.72E–01	0.2333	–5.0853	5.5518	9.30E–01
HDL L PN (nmol·L^{-1})	6.6720	–33.3875	46.7316	7.40E–01	4.8238	–35.1531	44.8007	8.10E–01
HDL M PN (nmol·L^{-1})	–6.9981	–43.5765	29.5802	7.03E–01	–9.3789	–45.9249	27.1672	6.09E–01
HDL S PN (nmol·L^{-1})	–2.5932	–33.9902	28.8037	8.69E–01	–1.3061	–33.1582	30.5460	9.35E–01
HDL VS PN (nmol·L^{-1})	2.0747	–13.7713	17.9207	7.94E–01	2.9408	–13.2640	19.1457	7.18E–01
CM C (mmol·L^{-1})	0.0003	–0.0003	0.0009	2.95E–01	0.0004	–0.0002	0.0010	1.85E–01
VLDL C (mmol·L^{-1})	–0.0051	–0.0181	0.0079	4.36E–01	–0.0045	–0.0182	0.0091	5.07E–01
VLDL L1 C (mmol·L^{-1})	0.0001	–0.0006	0.0008	7.62E–01	0.0003	–0.0006	0.0011	5.37E–01
VLDL L2 C (mmol·L^{-1})	0.0004	–0.0015	0.0022	6.69E–01	0.0007	–0.0013	0.0028	4.83E–01
VLDL L3 C (mmol·L^{-1})	–0.0014	–0.0054	0.0027	5.11E–01	–0.0010	–0.0055	0.0034	6.42E–01
VLDL M C (mmol·L^{-1})	–0.0018	–0.0068	0.0031	4.60E–01	–0.0016	–0.0067	0.0034	5.19E–01
VLDL S C (mmol·L^{-1})	–0.0018	–0.0051	0.0015	2.81E–01	–0.0018	–0.0051	0.0016	2.89E–01
LDL C (mmol·L^{-1})	0.0014	–0.0202	0.0231	8.97E–01	0.0018	–0.0201	0.0237	8.69E–01
LDL L C (mmol·L^{-1})	–0.0021	–0.0098	0.0056	5.90E–01	–0.0020	–0.0097	0.0058	6.09E–01
LDL M C (mmol·L^{-1})	0.0010	–0.0092	0.0111	8.51E–01	0.0012	–0.0091	0.0114	8.21E–01
LDL S C (mmol·L^{-1})	0.0008	–0.0036	0.0052	7.18E–01	0.0009	–0.0035	0.0054	6.79E–01
LDL VS C (mmol·L^{-1})	0.0001	–0.0016	0.0017	9.36E–01	0.0001	–0.0015	0.0018	8.93E–01
HDL C (mmol·L^{-1})	0.0022	–0.0141	0.0184	7.89E–01	0.0012	–0.0150	0.0174	8.80E–01
HDL VL C (mmol·L^{-1})	0.0001	–0.0018	0.0021	8.90E–01	0.0001	–0.0019	0.0020	9.56E–01
HDL L C (mmol·L^{-1})	0.0014	–0.0071	0.0099	7.44E–01	0.0010	–0.0075	0.0095	8.18E–01
HDL M C (mmol·L^{-1})	–0.0006	–0.0056	0.0045	8.23E–01	–0.0010	–0.0061	0.0041	7.07E–01
HDL S C (mmol·L^{-1})	–0.0001	–0.0025	0.0022	9.14E–01	–0.0001	–0.0024	0.0023	9.58E–01

HDL VS C (mmol·L^{-1})	0.0001	–0.0008	0.0009	8.53E–01	0.0001	–0.0007	0.0010	7.96E–01
Total C (mmol·L^{-1})	–0.0034	–0.0337	0.0270	8.25E–01	–0.0030	–0.0337	0.0276	8.44E–01
Non-HDL C (mmol·L^{-1})	–0.0031	–0.0273	0.0212	8.01E–01	–0.0026	–0.0276	0.0223	8.32E–01
CM TG (mmol·L^{-1})	0.0012	–0.0005	0.0029	1.78E–01	0.0015	–0.0003	0.0033	9.78E–02
VLDL TG (mmol·L^{-1})	0.0026	–0.0113	0.0166	7.08E–01	0.0053	–0.0103	0.0209	4.98E–01
VLDL L1 TG (mmol·L^{-1})	0.0012	–0.0008	0.0032	2.22E–01	0.0017	–0.0005	0.0039	1.25E–01
VLDL L2 TG (mmol·L^{-1})	0.0021	–0.0019	0.0062	3.01E–01	0.0031	–0.0015	0.0076	1.83E–01
VLDL L3 TG (mmol·L^{-1})	0.0009	–0.0042	0.0060	7.19E–01	0.0019	–0.0038	0.0076	5.08E–01
VLDL M TG (mmol·L^{-1})	–0.0004	–0.0032	0.0025	8.07E–01	0.0000	–0.0031	0.0031	9.89E–01
VLDL S TG (mmol·L^{-1})	–0.0007	–0.0017	0.0004	2.01E–01	–0.0006	–0.0017	0.0004	2.42E–01
LDL TG (mmol·L^{-1})	–0.0013	–0.0033	0.0008	2.15E–01	–0.0012	–0.0033	0.0008	2.41E–01
LDL L TG (mmol·L^{-1})	–0.0008	–0.0021	0.0004	1.89E–01	–0.0008	–0.0021	0.0005	2.07E–01
LDL M TG (mmol·L^{-1})	–0.0004	–0.0012	0.0004	3.48E–01	–0.0004	–0.0012	0.0005	3.65E–01
LDL S TG (mmol·L^{-1})	0.0001	–0.0002	0.0005	3.76E–01	0.0002	–0.0002	0.0005	3.00E–01
LDL VS TG (mmol·L^{-1})	0.0001	–0.0001	0.0002	3.83E–01	0.0001	–0.0001	0.0002	2.71E–01
HDL TG (mmol·L^{-1})	–0.0006	–0.0029	0.0017	6.12E–01	–0.0005	–0.0028	0.0019	6.75E–01
HDL VL TG (mmol·L^{-1})	0.0000	–0.0002	0.0001	6.69E–01	0.0000	–0.0002	0.0001	6.30E–01
HDL L TG (mmol·L^{-1})	–0.0002	–0.0009	0.0004	5.23E–01	–0.0002	–0.0009	0.0004	4.55E–01
HDL M TG (mmol·L^{-1})	–0.0002	–0.0012	0.0007	6.09E–01	–0.0002	–0.0012	0.0008	7.02E–01
HDL S TG (mmol·L^{-1})	–0.0001	–0.0007	0.0005	7.60E–01	0.0000	–0.0006	0.0006	9.27E–01
HDL VS TG (mmol·L^{-1})	0.0000	–0.0001	0.0002	4.81E–01	0.0001	–0.0001	0.0002	2.90E–01
Total TG (mmol·L^{-1})	0.0034	–0.0141	0.0210	6.98E–01	0.0066	–0.0129	0.0261	4.98E–01
VLDL size (nm)	0.0876	–0.0400	0.2152	1.74E–01	0.1185	–0.0210	0.2580	9.45E–02
LDL size (nm)	–0.0062	–0.0166	0.0042	2.39E–01	–0.0068	–0.0172	0.0035	1.92E–01
HDL size (nm)	0.0005	–0.0098	0.0108	9.20E–01	–0.0001	–0.0104	0.0102	9.82E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 8. Associations between change in moderate-intensity physical activity (MPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0106	–0.0157	0.0369	4.22E–01	0.0145	–0.0114	0.0405	2.67E–01
VLDL L1 PN (nmol·L^{-1})	0.0111	–0.0692	0.0914	7.82E–01	0.0254	–0.0542	0.1051	5.25E–01
VLDL L2 PN (nmol·L^{-1})	0.0350	–0.2491	0.3192	8.06E–01	0.0881	–0.1937	0.3699	5.34E–01
VLDL L3 PN (nmol·L^{-1})	–0.2306	–0.8695	0.4083	4.73E–01	–0.1223	–0.7639	0.5193	7.04E–01
VLDL M PN (nmol·L^{-1})	–0.5630	–1.3596	0.2336	1.62E–01	–0.4670	–1.2577	0.3237	2.42E–01
VLDL S PN (nmol·L^{-1})	–0.9850	–1.6634	–0.3065	5.20E–03	–0.9667	–1.6438	–0.2896	5.94E–03
LDL L PN (nmol·L^{-1})	–1.1913	–3.6290	1.2463	3.32E–01	–1.1550	–3.5964	1.2865	3.47E–01
LDL M PN (nmol·L^{-1})	2.5004	–3.3957	8.3966	3.99E–01	2.6286	–3.2097	8.4669	3.71E–01
LDL S PN (nmol·L^{-1})	1.8869	–1.1943	4.9681	2.25E–01	1.9861	–1.0460	5.0182	1.95E–01
LDL VS PN (nmol·1^{-1})	0.5127	–0.9877	2.0130	4.96E–01	0.5580	–0.9190	2.0351	4.52E–01
HDL VL PN (nmol·L^{-1})	–0.7404	–6.0243	4.5436	7.80E–01	–1.0911	–6.3625	4.1802	6.80E–01
HDL L PN (nmol·L^{-1})	2.1702	–37.5730	41.9133	9.13E–01	–0.9257	–40.6145	38.7632	9.63E–01
HDL M PN (nmol·L^{-1})	–20.0602	–57.0219	16.9015	2.82E–01	–23.0438	–60.2602	14.1726	2.20E–01
HDL S PN (nmol·L^{-1})	4.4812	–34.2539	43.2162	8.18E–01	6.1830	–32.6127	44.9787	7.51E–01
HDL VS PN (nmol·L^{-1})	10.9407	–6.8116	28.6931	2.22E–01	11.9216	–5.5045	29.3477	1.76E–01
CM C (mmol·L^{-1})	0.0001	–0.0009	0.0011	8.40E–01	0.0002	–0.0007	0.0012	6.17E–01
VLDL C (mmol·L^{-1})	–0.0138	–0.0265	–0.0011	3.41E–02	–0.0127	–0.0256	0.0002	5.35E–02
VLDL L1 C (mmol·L^{-1})	–0.0001	–0.0013	0.0010	7.95E–01	0.0000	–0.0011	0.0012	9.34E–01
VLDL L2 C (mmol·L^{-1})	–0.0005	–0.0032	0.0022	7.17E–01	–0.0001	–0.0028	0.0026	9.66E–01
VLDL L3 C (mmol·L^{-1})	–0.0031	–0.0078	0.0015	1.78E–01	–0.0027	–0.0073	0.0020	2.62E–01
VLDL M C (mmol·L^{-1})	–0.0043	–0.0077	–0.0009	1.36E–02	–0.0040	–0.0074	–0.0005	2.42E–02
VLDL S C (mmol·L^{-1})	–0.0040	–0.0071	–0.0009	1.12E–02	–0.0040	–0.0070	–0.0009	1.19E–02
LDL C (mmol·L^{-1})	0.0112	–0.0172	0.0395	4.33E–01	0.0118	–0.0163	0.0399	4.03E–01
LDL L C (mmol·L^{-1})	–0.0023	–0.0113	0.0067	6.08E–01	–0.0022	–0.0112	0.0068	6.24E–01
LDL M C (mmol·L^{-1})	0.0071	–0.0064	0.0205	2.96E–01	0.0074	–0.0060	0.0207	2.73E–01
LDL S C (mmol·L^{-1})	0.0041	–0.0017	0.0098	1.60E–01	0.0042	–0.0014	0.0098	1.35E–01
LDL VS C (mmol·L^{-1})	0.0010	–0.0011	0.0032	3.34E–01	0.0011	–0.0010	0.0032	2.95E–01
HDL C (mmol·L^{-1})	0.0006	–0.0149	0.0160	9.40E–01	–0.0010	–0.0166	0.0147	9.03E–01
HDL VL C (mmol·L^{-1})	–0.0004	–0.0024	0.0016	6.87E–01	–0.0006	–0.0026	0.0014	5.81E–01
HDL L C (mmol·L^{-1})	–0.0007	–0.0097	0.0083	8.71E–01	–0.0015	–0.0105	0.0076	7.45E–01
HDL M C (mmol·L^{-1})	–0.0010	–0.0059	0.0039	6.71E–01	–0.0015	–0.0065	0.0034	5.36E–01
HDL S C (mmol·L^{-1})	0.0010	–0.0018	0.0037	4.81E–01	0.0011	–0.0017	0.0038	4.43E–01

HDL VS C (mmol·L^{-1})	0.0006	–0.0004	0.0015	2.20E–01	0.0006	–0.0003	0.0015	1.85E–01
Total C (mmol·L^{-1})	–0.0079	–0.0445	0.0286	6.65E–01	–0.0075	–0.0440	0.0290	6.82E–01
Non-HDL C (mmol·L^{-1})	–0.0072	–0.0387	0.0243	6.49E–01	–0.0063	–0.0379	0.0253	6.89E–01
CM TG (mmol·L^{-1})	0.0012	–0.0019	0.0042	4.51E–01	0.0016	–0.0014	0.0046	2.96E–01
VLDL TG (mmol·L^{-1})	–0.0032	–0.0237	0.0173	7.54E–01	0.0004	–0.0199	0.0207	9.66E–01
VLDL L1 TG (mmol·L^{-1})	0.0008	–0.0027	0.0044	6.45E–01	0.0014	–0.0021	0.0049	4.23E–01
VLDL L2 TG (mmol·L^{-1})	0.0008	–0.0058	0.0075	8.00E–01	0.0020	–0.0046	0.0087	5.37E–01
VLDL L3 TG (mmol·L^{-1})	–0.0012	–0.0084	0.0061	7.47E–01	0.0002	–0.0070	0.0074	9.62E–01
VLDL M TG (mmol·L^{-1})	–0.0018	–0.0051	0.0014	2.60E–01	–0.0014	–0.0045	0.0018	3.92E–01
VLDL S TG (mmol·L^{-1})	–0.0012	–0.0021	–0.0003	8.12E–03	–0.0012	–0.0020	–0.0003	1.11E–02
LDL TG (mmol·L^{-1})	–0.0021	–0.0043	0.0001	6.42E–02	–0.0020	–0.0042	0.0002	7.42E–02
LDL L TG (mmol·L^{-1})	–0.0014	–0.0025	–0.0004	8.51E–03	–0.0014	–0.0024	–0.0003	1.04E–02
LDL M TG (mmol·L^{-1})	–0.0006	–0.0015	0.0003	1.75E–01	–0.0006	–0.0015	0.0003	1.85E–01
LDL S TG (mmol·L^{-1})	0.0001	–0.0004	0.0006	6.29E–01	0.0001	–0.0003	0.0006	5.40E–01
LDL VS TG (mmol·L^{-1})	0.0000	–0.0002	0.0003	7.42E–01	0.0001	–0.0002	0.0003	5.97E–01
HDL TG (mmol·L^{-1})	–0.0021	–0.0048	0.0005	1.12E–01	–0.0020	–0.0047	0.0006	1.31E–01
HDL VL TG (mmol·L^{-1})	–0.0002	–0.0004	0.0000	8.13E–02	–0.0002	–0.0004	0.0000	7.83E–02
HDL L TG (mmol·L^{-1})	–0.0008	–0.0015	0.0000	4.85E–02	–0.0008	–0.0016	0.0000	3.81E–02
HDL M TG (mmol·L^{-1})	–0.0007	–0.0018	0.0003	1.75E–01	–0.0007	–0.0017	0.0004	2.15E–01
HDL S TG (mmol·L^{-1})	–0.0003	–0.0010	0.0004	4.59E–01	–0.0002	–0.0008	0.0005	6.21E–01
HDL VS TG (mmol·L^{-1})	0.0000	–0.0002	0.0002	7.61E–01	0.0001	–0.0001	0.0003	4.95E–01
Total TG (mmol·L^{-1})	–0.0044	–0.0315	0.0227	7.47E–01	–0.0001	–0.0269	0.0267	9.94E–01
VLDL size (nm)	0.0757	–0.1280	0.2793	4.60E–01	0.1134	–0.0892	0.3161	2.67E–01
LDL size (nm)	–0.0138	–0.0228	–0.0048	3.20E–03	–0.0144	–0.0233	–0.0056	1.92E–03
HDL size (nm)	–0.0038	–0.0154	0.0079	5.21E–01	–0.0048	–0.0165	0.0069	4.14E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 9. Associations between change in light-intensity physical activity (LPA) and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	0.0154	–0.0195	0.0503	3.82E–01	0.0211	–0.0135	0.0556	2.27E–01
VLDL L1 PN (nmol·L^{-1})	0.0163	–0.0842	0.1169	7.46E–01	0.0364	–0.0631	0.1360	4.66E–01
VLDL L2 PN (nmol·L^{-1})	0.0063	–0.3423	0.3549	9.71E–01	0.0813	–0.2627	0.4254	6.38E–01
VLDL L3 PN (nmol·L^{-1})	–0.2353	–0.9772	0.5067	5.28E–01	–0.0865	–0.8080	0.6350	8.11E–01
VLDL M PN (nmol·L^{-1})	–0.4388	–1.4030	0.5254	3.66E–01	–0.2843	–1.1942	0.6256	5.34E–01
VLDL S PN (nmol·L^{-1})	–0.3548	–1.1115	0.4019	3.52E–01	–0.3149	–1.0358	0.4060	3.85E–01
LDL L PN (nmol·L^{-1})	–0.5804	–2.9999	1.8390	6.33E–01	–0.4907	–2.8443	1.8628	6.78E–01
LDL M PN (nmol·L^{-1})	0.0889	–5.4470	5.6248	9.74E–01	0.4230	–5.1360	5.9820	8.79E–01
LDL S PN (nmol·L^{-1})	0.1158	–3.0951	3.3267	9.43E–01	0.3603	–2.8580	3.5785	8.23E–01
LDL VS PN (nmol·1^{-1})	0.0616	–1.4823	1.6055	9.37E–01	0.1714	–1.3817	1.7246	8.26E–01
HDL VL PN (nmol·L^{-1})	1.8640	–3.4811	7.2091	4.88E–01	1.3078	–3.8547	6.4703	6.14E–01
HDL L PN (nmol·L^{-1})	12.2894	–29.2411	53.8199	5.56E–01	7.8932	–32.6521	48.4384	6.98E–01
HDL M PN (nmol·L^{-1})	12.5207	–25.9716	51.0130	5.17E–01	7.4208	–31.4532	46.2948	7.04E–01
HDL S PN (nmol·L^{-1})	1.4009	–36.7522	39.5539	9.42E–01	4.5969	–33.2153	42.4090	8.08E–01
HDL VS PN (nmol·L^{-1})	–4.2003	–23.8743	15.4736	6.71E–01	–2.2451	–21.5280	17.0378	8.16E–01
CM C (mmol·L^{-1})	0.0004	–0.0009	0.0016	5.61E–01	0.0006	–0.0006	0.0018	3.46E–01
VLDL C (mmol·L^{-1})	0.0007	–0.0142	0.0156	9.22E–01	0.0024	–0.0121	0.0169	7.41E–01
VLDL L1 C (mmol·L^{-1})	0.0001	–0.0013	0.0014	9.25E–01	0.0003	–0.0010	0.0016	6.18E–01
VLDL L2 C (mmol·L^{-1})	–0.0001	–0.0033	0.0031	9.70E–01	0.0006	–0.0025	0.0037	7.12E–01
VLDL L3 C (mmol·L^{-1})	–0.0020	–0.0074	0.0033	4.53E–01	–0.0013	–0.0066	0.0041	6.39E–01
VLDL M C (mmol·L^{-1})	0.0008	–0.0038	0.0053	7.30E–01	0.0013	–0.0031	0.0057	5.51E–01
VLDL S C (mmol·L^{-1})	–0.0009	–0.0041	0.0023	5.63E–01	–0.0008	–0.0039	0.0022	5.80E–01
LDL C (mmol·L^{-1})	0.0016	–0.0243	0.0276	9.00E–01	0.0032	–0.0227	0.0291	8.05E–01
LDL L C (mmol·L^{-1})	–0.0030	–0.0119	0.0059	4.99E–01	–0.0028	–0.0114	0.0058	5.18E–01
LDL M C (mmol·L^{-1})	–0.0004	–0.0127	0.0119	9.44E–01	0.0003	–0.0119	0.0126	9.59E–01
LDL S C (mmol·L^{-1})	0.0004	–0.0053	0.0061	8.93E–01	0.0009	–0.0048	0.0065	7.64E–01
LDL VS C (mmol·L^{-1})	0.0001	–0.0020	0.0022	8.95E–01	0.0003	–0.0018	0.0024	7.72E–01
HDL C (mmol·L^{-1})	0.0063	–0.0086	0.0212	3.99E–01	0.0041	–0.0106	0.0188	5.80E–01
HDL VL C (mmol·L^{-1})	0.0009	–0.0010	0.0029	3.38E–01	0.0007	–0.0012	0.0026	4.50E–01
HDL L C (mmol·L^{-1})	0.0028	–0.0061	0.0117	5.29E–01	0.0018	–0.0069	0.0104	6.80E–01
HDL M C (mmol·L^{-1})	0.0030	–0.0016	0.0075	1.97E–01	0.0022	–0.0024	0.0067	3.44E–01
HDL S C (mmol·L^{-1})	–0.0002	–0.0028	0.0023	8.57E–01	–0.0001	–0.0026	0.0025	9.60E–01

HDL VS C (mmol·L^{-1})	–0.0002	–0.0012	0.0007	6.39E–01	–0.0002	–0.0011	0.0008	7.53E–01
Total C (mmol·L^{-1})	0.0084	–0.0259	0.0427	6.25E–01	0.0095	–0.0247	0.0437	5.79E–01
Non-HDL C (mmol·L^{-1})	0.0044	–0.0259	0.0348	7.70E–01	0.0064	–0.0239	0.0366	6.75E–01
CM TG (mmol·L^{-1})	0.0021	–0.0020	0.0061	3.09E–01	0.0027	–0.0013	0.0067	1.79E–01
VLDL TG (mmol·L^{-1})	–0.0021	–0.0264	0.0222	8.63E–01	0.0030	–0.0207	0.0267	8.00E–01
VLDL L1 TG (mmol·L^{-1})	0.0014	–0.0030	0.0058	5.33E–01	0.0022	–0.0021	0.0066	3.11E–01
VLDL L2 TG (mmol·L^{-1})	0.0008	–0.0072	0.0088	8.47E–01	0.0025	–0.0054	0.0104	5.31E–01
VLDL L3 TG (mmol·L^{-1})	–0.0016	–0.0101	0.0069	7.03E–01	0.0003	–0.0080	0.0085	9.52E–01
VLDL M TG (mmol·L^{-1})	–0.0021	–0.0059	0.0017	2.78E–01	–0.0014	–0.0050	0.0023	4.54E–01
VLDL S TG (mmol·L^{-1})	–0.0006	–0.0016	0.0005	2.84E–01	–0.0005	–0.0015	0.0006	3.71E–01
LDL TG (mmol·L^{-1})	0.0008	–0.0023	0.0038	6.24E–01	0.0009	–0.0022	0.0040	5.74E–01
LDL L TG (mmol·L^{-1})	–0.0002	–0.0014	0.0011	7.93E–01	–0.0001	–0.0013	0.0012	8.82E–01
LDL M TG (mmol·L^{-1})	0.0004	–0.0008	0.0015	5.36E–01	0.0004	–0.0008	0.0016	5.13E–01
LDL S TG (mmol·L^{-1})	0.0004	–0.0003	0.0010	2.46E–01	0.0004	–0.0002	0.0010	1.82E–01
LDL VS TG (mmol·L^{-1})	0.0002	–0.0002	0.0005	3.32E–01	0.0002	–0.0001	0.0005	2.22E–01
HDL TG (mmol·L^{-1})	0.0005	–0.0027	0.0038	7.42E–01	0.0008	–0.0024	0.0039	6.36E–01
HDL VL TG (mmol·L^{-1})	0.0001	–0.0001	0.0003	3.41E–01	0.0001	–0.0001	0.0003	4.03E–01
HDL L TG (mmol·L^{-1})	0.0003	–0.0005	0.0011	5.12E–01	0.0002	–0.0007	0.0010	6.67E–01
HDL M TG (mmol·L^{-1})	0.0001	–0.0012	0.0015	8.65E–01	0.0002	–0.0010	0.0015	7.07E–01
HDL S TG (mmol·L^{-1})	0.0001	–0.0008	0.0010	8.26E–01	0.0002	–0.0006	0.0010	5.89E–01
HDL VS TG (mmol·L^{-1})	0.0001	–0.0002	0.0003	6.62E–01	0.0001	–0.0001	0.0003	3.92E–01
Total TG (mmol·L^{-1})	0.0016	–0.0312	0.0343	9.25E–01	0.0077	–0.0241	0.0394	6.31E–01
VLDL size (nm)	0.0503	–0.2013	0.3019	6.90E–01	0.1026	–0.1473	0.3524	4.14E–01
LDL size (nm)	–0.0041	–0.0162	0.0081	5.04E–01	–0.0053	–0.0171	0.0065	3.73E–01
HDL size (nm)	0.0039	–0.0081	0.0158	5.21E–01	0.0024	–0.0092	0.0140	6.83E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 10. Associations between change in sedentary time and follow-up lipoprotein measures

Lipoprotein measure	Model 1				Model 2			
	Coefficient	Lower CI	Upper CI	*p* value	Coefficient	Lower CI	Upper CI	*p* value
CM PN (nmol·L^{-1})	–0.0208	–0.0572	0.0156	2.57E–01	–0.0280	–0.0650	0.0089	1.34E–01
VLDL L1 PN (nmol·L^{-1})	–0.0278	–0.1329	0.0773	5.98E–01	–0.0533	–0.1609	0.0544	3.26E–01
VLDL L2 PN (nmol·L^{-1})	–0.0632	–0.4262	0.2999	7.29E–01	–0.1581	–0.5306	0.2144	3.99E–01
VLDL L3 PN (nmol·L^{-1})	0.2758	–0.5123	1.0640	4.86E–01	0.0910	–0.7109	0.8930	8.21E–01
VLDL M PN (nmol·L^{-1})	0.5925	–0.4723	1.6572	2.70E–01	0.4134	–0.6324	1.4593	4.32E–01
VLDL S PN (nmol·L^{-1})	0.8479	0.0131	1.6826	4.66E–02	0.8078	–0.0024	1.6181	5.07E–02
LDL L PN (nmol·L^{-1})	1.1970	–1.3815	3.7754	3.56E–01	1.1072	–1.4480	3.6623	3.89E–01
LDL M PN (nmol·L^{-1})	–1.1072	–7.2414	5.0271	7.19E–01	–1.4464	–7.6239	4.7311	6.41E–01
LDL S PN (nmol·L^{-1})	–0.8629	–4.4405	2.7146	6.31E–01	–1.1161	–4.7132	2.4809	5.37E–01
LDL VS PN (nmol·1^{-1})	–0.2108	–1.9278	1.5061	8.07E–01	–0.3254	–2.0565	1.4057	7.08E–01
HDL VL PN (nmol·L^{-1})	–1.2934	–7.6965	5.1097	6.87E–01	–0.6608	–6.8660	5.5444	8.32E–01
HDL L PN (nmol·L^{-1})	–12.8189	–60.9713	35.3335	5.96E–01	–7.5945	–54.1897	39.0006	7.45E–01
HDL M PN (nmol·L^{-1})	0.6975	–41.5661	42.9611	9.74E–01	6.7164	–35.3489	48.7818	7.50E–01
HDL S PN (nmol·L^{-1})	–2.0608	–41.7468	37.6252	9.18E–01	–5.6777	–45.8857	34.5303	7.78E–01
HDL VS PN (nmol·L^{-1})	–1.6689	–22.5853	19.2476	8.74E–01	–3.9137	–24.8108	16.9835	7.09E–01
CM C (mmol·L^{-1})	–0.0004	–0.0017	0.0008	4.86E–01	–0.0007	–0.0020	0.0006	2.76E–01
VLDL C (mmol·L^{-1})	0.0069	–0.0090	0.0228	3.87E–01	0.0050	–0.0109	0.0209	5.30E–01
VLDL L1 C (mmol·L^{-1})	0.0000	–0.0015	0.0014	9.52E–01	–0.0004	–0.0018	0.0011	6.03E–01
VLDL L2 C (mmol·L^{-1})	0.0000	–0.0034	0.0035	9.82E–01	–0.0008	–0.0042	0.0027	6.66E–01
VLDL L3 C (mmol·L^{-1})	0.0034	–0.0022	0.0090	2.24E–01	0.0025	–0.0032	0.0083	3.85E–01
VLDL M C (mmol·L^{-1})	0.0018	–0.0027	0.0064	4.18E–01	0.0012	–0.0032	0.0057	5.82E–01
VLDL S C (mmol·L^{-1})	0.0030	–0.0007	0.0067	1.14E–01	0.0029	–0.0007	0.0065	1.12E–01
LDL C (mmol·L^{-1})	–0.0056	–0.0346	0.0233	6.98E–01	–0.0072	–0.0364	0.0219	6.21E–01
LDL L C (mmol·L^{-1})	0.0041	–0.0055	0.0137	3.96E–01	0.0038	–0.0056	0.0133	4.17E–01
LDL M C (mmol·L^{-1})	–0.0025	–0.0162	0.0112	7.14E–01	–0.0033	–0.0171	0.0105	6.34E–01
LDL S C (mmol·L^{-1})	–0.0020	–0.0084	0.0043	5.28E–01	–0.0025	–0.0089	0.0039	4.36E–01
LDL VS C (mmol·L^{-1})	–0.0005	–0.0028	0.0019	6.88E–01	–0.0006	–0.0030	0.0017	5.86E–01
HDL C (mmol·L^{-1})	–0.0060	–0.0238	0.0118	5.04E–01	–0.0033	–0.0208	0.0141	7.03E–01
HDL VL C (mmol·L^{-1})	–0.0006	–0.0030	0.0018	6.03E–01	–0.0004	–0.0027	0.0019	7.57E–01
HDL L C (mmol·L^{-1})	–0.0025	–0.0133	0.0084	6.50E–01	–0.0012	–0.0118	0.0093	8.13E–01
HDL M C (mmol·L^{-1})	–0.0016	–0.0067	0.0035	5.24E–01	–0.0007	–0.0057	0.0044	7.89E–01
HDL S C (mmol·L^{-1})	–0.0001	–0.0029	0.0027	9.20E–01	–0.0003	–0.0031	0.0025	8.16E–01

HDL VS C (mmol·L^{-1})	–0.0001	–0.0012	0.0010	9.08E–01	–0.0002	–0.0012	0.0009	7.82E–01
Total C (mmol·L^{-1})	–0.0015	–0.0415	0.0385	9.39E–01	–0.0027	–0.0430	0.0376	8.95E–01
Non-HDL C (mmol·L^{-1})	0.0011	–0.0322	0.0344	9.48E–01	–0.0008	–0.0347	0.0330	9.61E–01
CM TG (mmol·L^{-1})	–0.0026	–0.0068	0.0016	2.27E–01	–0.0034	–0.0076	0.0009	1.18E–01
VLDL TG (mmol·L^{-1})	0.0015	–0.0239	0.0269	9.07E–01	–0.0049	–0.0308	0.0210	7.05E–01
VLDL L1 TG (mmol·L^{-1})	–0.0019	–0.0065	0.0027	4.02E–01	–0.0030	–0.0077	0.0017	2.05E–01
VLDL L2 TG (mmol·L^{-1})	–0.0019	–0.0103	0.0065	6.51E–01	–0.0041	–0.0127	0.0046	3.49E–01
VLDL L3 TG (mmol·L^{-1})	0.0012	–0.0077	0.0101	7.82E–01	–0.0011	–0.0102	0.0080	8.06E–01
VLDL M TG (mmol·L^{-1})	0.0024	–0.0017	0.0065	2.42E–01	0.0015	–0.0025	0.0056	4.48E–01
VLDL S TG (mmol·L^{-1})	0.0012	0.0000	0.0023	4.60E–02	0.0011	–0.0001	0.0022	6.81E–02
LDL TG (mmol·L^{-1})	0.0007	–0.0026	0.0041	6.63E–01	0.0006	–0.0028	0.0040	7.23E–01
LDL L TG (mmol·L^{-1})	0.0010	–0.0004	0.0024	1.56E–01	0.0009	–0.0005	0.0024	1.89E–01
LDL M TG (mmol·L^{-1})	0.0001	–0.0012	0.0014	8.60E–01	0.0001	–0.0013	0.0014	8.95E–01
LDL S TG (mmol·L^{-1})	–0.0004	–0.0010	0.0003	2.44E–01	–0.0005	–0.0011	0.0002	1.80E–01
LDL VS TG (mmol·L^{-1})	–0.0002	–0.0005	0.0002	3.30E–01	–0.0002	–0.0006	0.0001	2.15E–01
HDL TG (mmol·L^{-1})	0.0006	–0.0030	0.0042	7.39E–01	0.0004	–0.0032	0.0039	8.42E–01
HDL VL TG (mmol·L^{-1})	0.0000	–0.0002	0.0002	9.96E–01	0.0000	–0.0002	0.0003	9.26E–01
HDL L TG (mmol·L^{-1})	0.0002	–0.0008	0.0011	7.15E–01	0.0003	–0.0007	0.0012	5.69E–01
HDL M TG (mmol·L^{-1})	0.0003	–0.0012	0.0017	7.12E–01	0.0001	–0.0013	0.0015	8.64E–01
HDL S TG (mmol·L^{-1})	0.0001	–0.0009	0.0010	9.12E–01	–0.0001	–0.0010	0.0008	8.17E–01
HDL VS TG (mmol·L^{-1})	–0.0001	–0.0003	0.0002	5.78E–01	–0.0001	–0.0004	0.0001	3.00E–01
Total TG (mmol·L^{-1})	–0.0013	–0.0358	0.0331	9.39E–01	–0.0090	–0.0438	0.0258	6.07E–01
VLDL size (nm)	–0.1062	–0.3673	0.1549	4.19E–01	–0.1738	–0.4433	0.0956	2.02E–01
LDL size (nm)	0.0107	–0.0029	0.0243	1.20E–01	0.0122	–0.0011	0.0254	7.17E–02
HDL size (nm)	–0.0017	–0.0154	0.0119	8.00E–01	0.0000	–0.0131	0.0132	9.95E–01

Regression coefficients are in absolute concentration units of lipoprotein measures per SD unit increment of change in activity variable (follow-up minus baseline).
Model 1 is adjusted for change in accelerometer wear time, and baseline values of age, lipoprotein measure, parents' education, sex, and sexual maturity. Cluster-robust standard errors were calculated, clustered on the school variable.
Model 2 is adjusted for baseline waist circumference in addition to the Model 1 covariates.
p values should be interpreted at a Bonferroni-corrected threshold of 0.01.
In notation of *p* values 1.23E–02 stands for "1.23 times 10 to the power of –02" or 0.0123.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Paper IV

Moderation of the association between aerobic fitness and lipoprotein subclass particle numbers by moderate- to vigorous-intensity physical activity: a prospective cohort study.

Abstract

Background: Circulating concentrations (particle numbers) of various lipoprotein subclasses are associated with aerobic fitness and physical activity (PA). That these associations are independent of the other exposure has not been investigated. We investigated whether daily moderate- to vigorous-intensity physical activity (MVPA) moderates the associations between aerobic fitness and lipoprotein subclass particle numbers and if adiposity confounds these associations.

Methods: We included 773 fifth grade (mean [SD] age = 10.0 [0.3] y) Norwegian schoolchildren (50.2% girls) measured on two separate occasions across one academic year. Our main exposure was aerobic fitness measured using an intermittent shuttle run test. Daily MVPA was recorded by triaxial accelerometers worn for seven consecutive days. Outcome variables were the particle numbers for 15 lipoprotein subclasses calculated from targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy profiles of fasted serum blood samples. We used separate multivariable linear regression models to analyse prospective associations between aerobic fitness and each lipoprotein variable and included an interaction term (aerobic fitness x MVPA) to examine potential effect modification. All models were adjusted for baseline measures of the respective lipoprotein variable, age, parents' education, school, sex, and sexual maturity. We repeated the analysis of each model additionally adjusting for waist circumference.

Results: There were interactions between aerobic fitness and daily MVPA for the larger, triglyceride-rich lipoprotein subclasses (e.g., $p = 0.001$ for the VLDL L1 subclass). We probed these interactions by examining the association trends at three different levels of daily MVPA (mean, mean ± 1 SD). For the same triglyceride-rich lipoprotein subclasses we found evidence of effect modification at the higher level of MVPA, which was more marked towards the lower end of the aerobic fitness distribution.

Conclusion: We report evidence that daily MVPA moderates the associations between aerobic fitness and particle numbers of certain lipoprotein subclasses. This influence is more pronounced at lower levels of fitness.

1. Introduction

Aerobic fitness is a physiological measure of the ability to deliver oxygen to mitochondria during physical work and is considered a reflection of total body health.[1] Physical activity (PA) is a behaviour and refers to any bodily movement produced by skeletal muscles that expends energy.[2] Both are consistently associated with a number of health outcomes in adults, including inverse associations with all-cause and cardiovascular disease (CVD) mortality.[3,4] In children and adolescents, aerobic fitness and PA are beneficially associated with measures of lipoprotein metabolism thought indicative of cardiometabolic risk.[5,6] However, there is conflicting evidence regarding whether the associations between each exposure and cardiometabolic risk factors are independent of each other, whether there is effect modification on one exposure at different levels of the other, and to what extent adiposity attenuates these associations.[7,8]

Apolipoprotein B (ApoB) is the primary organising protein of chylomicron, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) particles, and is recognised as likely the causal trait through which these lipoproteins exert their influence on atherosclerotic cardiovascular disease (ASCVD) risk, rather than their lipid mass per se.[9,10] Given that each ApoB-containing lipoprotein carries a single ApoB molecule, it is thought that the circulating number/concentration of these particles contributes to their atherosclerotic potential, increasing the probability that they will enter and be retained in the arterial intima.[11,12] The recent advancement of quantitative high-throughput nuclear magnetic resonance (NMR) spectroscopy platforms has facilitated the analysis of a number of lipoprotein attributes not captured by the traditional lipid profile—such as various lipoprotein subclasses categorised by particle size—in large datasets.[13] Epidemiological analysis of children and young adults have shown that aerobic fitness and PA are individually associated with lower levels of ApoB-containing lipoproteins and that the strengths of these associations differ dependent on subclass.[14–16] These studies also reported divergent associations directions between high-density lipoprotein (HDL) subclasses.

In this study, we used targeted proton NMR (^{1}H NMR) spectroscopy to investigate possible effect modification of the prospective associations between aerobic fitness and lipoprotein subclass particle numbers by daily MVPA. We also examined these associations for potential confounding by adiposity.

2. Methods

Additional information regarding blood sample handling and the ^{1}H NMR protocol are reported in the Supplementary Material.

2.1. Sample population

We drew out analytical sample from the Active Smarter Kids (ASK) study. The ASK study was a seven-month cluster randomised controlled trial (RCT) to examine the effects of a school-based PA intervention on academic performance in fifth grade Norwegian schoolchildren, which commenced in 2014 (https://clinicaltrials.gov, #NCT02132494). The methods and analysis of primary outcomes have been reported previously.[17,18] Changes in physical activity levels and aerobic fitness were of a similar degree for children who either received the intervention or did not. We therefore pooled all children for this analysis.

2.2. Ethics

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.[19]

2.3. Exposure variable

We measured aerobic fitness using the Andersen intermittent shuttle run test.[20] The objective of the Andersen test is to cover the greatest distance possible in 10 minutes. Following an explanation of the test and five-minute warm-up, the children ran back and forth between two parallel lines set 20 metres apart, alternately running for 15 seconds then pausing for 15 seconds. Each time they reached either end line, the children had to touch beyond it with one finger before they could run back in the opposite direction. Study research assistants supervised testing and recorded the total distance (m) covered for each child. Whilst the Andersen test is a performance test, it 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 measure for peak $\dot{V}O_2$.[21] We used running distance as the main exposure as opposed to applying a prediction equation to convert distance covered to an estimate of peak $\dot{V}O_2$.

2.4. Moderator variable

The children wore hip-mounted accelerometers (ActiGraph GT3X+, ActiGraph LLC, Pensacola, FL) for seven consecutive days, except for when sleeping or during water-based activities. We defined a valid day as ≥480 min of monitor wear time between 0600 and 0000; non-wear time as ≥20 min of zero counts.[22] We analysed the accelerometer data using KineSoft software (version 3.3.80, KineSoft, Loughborough, United Kingdom), used 10-second epoch lengths, and classified moderate- to vigorous-intensity physical activity (MVPA) using the Evenson cut point of ≥2296 counts·min^{-1}.[23,24]

2.5. Outcome variables

Fasting serum samples were collected by either a trained nurse or phlebotomist between 0800 and 1000. We recorded NMR spectra on a Bruker Avance III 600 MHz spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). Spectral regions quantitatively associated to lipoprotein concentrations were selected as explanatory variables to calculate the particle numbers of 20 lipoprotein subclasses using partial least squares (PLS) modelling. We did this for 106 randomly selected serum samples analysed by both NMR and high-performance liquid chromatography (HPLC).[25] We used a Monte Carlo resampling approach to calculate individual PLS models with optimal prediction ability for the HPLC data.[26] Lipoprotein particle numbers for all samples were subsequently predicted from these models. We reduced the lipoprotein subclasses to 15 as previously described.[27] Given the elution of lipid-poor pre-β_1 HDLs, we only used the HDL6 subclass measure when calculating the particle number for the HDL VS subclass.[28] Also, though intact chylomicron particles cannot be distinguished from the largest VLDL particles, we have retained the nomenclature from the HPLC method which does distinguish the two. In our analysis, particles in the chylomicron class should be

considered either very large VLDLs. For a more detailed description of our ^{1}H NMR method, we refer readers to a previous publication.[14]

2.6. Anthropometrics

Each child had their height measured to the nearest 0.1 cm using a portable stadiometer (Seca 217, SECA GmbH, Hamburg, Germany), weight to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany), and waist circumference to the nearest 0.1 cm using a measuring tape (Seca 201, SECA GmbH, Hamburg, Germany). The children wore light clothing for the assessment, shoes removed. For waist circumference, we took two measurements between the lowest palpable rib and iliac crest, the child having gently exhaled. If there was a difference of more than 1.0 cm between the two measurements, a third was taken. The mean of the two measurements with the least difference was recorded for analysis. Body mass index (BMI) is the weight divided by the square of height ($kg \cdot m^{-2}$). We used the International Obesity Task Force's (IOTF) sex-specific BMI cut-off values to calculate the proportion of overweight and/or obese children, rounding the children's age down to the nearest half-year.[29]

2.7. Sexual maturity

Each child assessed their own sexual maturity against a standard set of images and text descriptions that correspond to the Tanner staging method.[30] The assessments took place in a private room and the children were accompanied by a researcher of the same sex to ensure their comfort. We recorded low frequencies of children in Tanner categories 3, 4, and 5 (n = 66, 5, 2, respectively of 1081 children with valid baseline data) and combined them into one category (≥3) for analysis.

2.8. Socioeconomic status

Parent(s) or guardian(s) individually completed a custom self-report study questionnaire, selecting their level of educational attainment from six categories. For analysis, we quantified socioeconomic status (SES) as the highest level of attainment of a child's mother, father, or guardian, whichever was higher. Given the low frequency of respondents that selected any of the lower four categories (n = 4, 15, 193, 137, for categories 1–4, respectively of 1069 children with valid baseline data), we combined them into one: *Upper secondary school*. Hence, we used three categories in our analysis.

2.9. Statistical approach

Prior to analysis, we converted all lipoprotein variables, aerobic fitness, and MVPA to z-scores (mean = 0.0; standard deviation [SD] = 1.0). We fitted separate prospective linear models for each lipoprotein subclass, regressing the follow-up particle number on aerobic fitness. Each model was adjusted for daily MVPA, particle number of the respective subclass at baseline, sex, parents' education, and baseline values of age and sexual maturity as fixed effects. To account for potential within-cluster correlation and to obviate the need to transform skewed outcome variables, we calculated cluster and heteroscedasticity robust standard errors clustered on school. We repeated the linear regression analysis including waist circumference as an additional covariate.

To examine whether MVPA moderated the effect of aerobic fitness on the lipoprotein measures, we added an interaction term (aerobic fitness x MVPA) to each model. We performed a *simple slopes* analysis, examining each regression model fixed at three sample-estimated values of MVPA: mean, and mean ± 1 SD.[31] The calculations were averaged over the levels of the categorical predictors: parents' education, sex, and sexual maturity. Mean values of the continuous covariates age and baseline lipoprotein variable were used. The simple slopes represent the change in the *z*-score lipoprotein variable per SD unit increase in aerobic fitness whilst holding MVPA constant at each of the three levels specified. We also examined these interactions visually, plotting the simple slopes at SD unit increments across the range of distances run in the Andersen test. We hypothesised that were MVPA to have a moderating effect on the association between aerobic fitness and lipoprotein subclass particle numbers it would be more marked at lower levels of fitness. Therefore, we performed pairwise comparisons of predicted values for each lipoprotein measure between ±1 SD of MVPA, at –1 SD of the Andersen test. We used principal component analysis (PCA) to estimate the effective number of independent tests to use for multiple testing correction. The rationale for this method has been described previously and applied in a number of metabolic profile studies.[16,32,33] In a previous analysis using *z*-scores of 57 lipoprotein measures, we calculated that 5 principal components explained >95% of the variance. Hence, our Bonferroni-corrected threshold for assessing associations is 0.05/5 = 0.01 (i.e., $p < 0.01$).

We conducted all analyses using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) within the RStudio integrated development environment version 1.1.456 (RStudio, PBC, Boston, MA).[34] 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 PCA analysis with **factoextra** (1.0.6) and the linear regression analysis using the **estimatr** (0.22.0) package, specifically the **lm_robust()** function, and used a number of functions within the **emmeans** (1.4.6) package in our analysis of trends and contrasts of predicted values. Plots were created using the **ggplot2** (3.3.0) package.

3. Results

3.1. Sample Characteristics

Our analytical sample comprised 773 children with complete data (50.2% girls) (Figure 1). The mean interval between baseline and follow-up blood sampling was 34.2 weeks. Of the total sample, 95.6% had at least four days of valid accelerometer data, and 50.4% had seven valid days. The correlation between aerobic fitness and daily MVPA was low to moderate (Pearson's $r = 0.39$, $p < 0.001$). Descriptive information for the analytical sample is given in Table 1. Means and SDs for the NMR lipoprotein measures are provided in Supplementary Material Table 1. Those children excluded due to missing data tended to be shorter, performed less daily MVPA, and achieved marginally less distance on the Andersen test, on average (Supplementary Material Table 2). A lower proportion were either overweight or obese. A higher proportion of boys than girls had missing data.

3.2. Interaction analysis

Aerobic fitness was prospectively associated with the particle numbers of the five largest VLDL subclasses (Table 2). All five associations were inverse (e.g., standardised coefficient

= –0.141; CI = –0.229, –0.053, p = 0.002 for VLDL L1). Having included an interaction term between aerobic fitness and daily MVPA, our results suggested a possible moderating role of daily MVPA for certain subclasses (Table 2). For three lipoprotein subclasses—chylomicrons, VLDL L1, and VLDL L2—the p value for the interaction term fell below the alpha level (e.g., standardised coefficient = 0.096; CI = 0.039, 0.152; p = 0.001 for VLDL L1). The main effects for aerobic fitness were very similar whether or not an interaction term was included (Supplementary Material Table 3).

3.3. Simple slopes (trends)

Examining the interaction plots suggested a differing effect of aerobic fitness on particle numbers dependent on daily MVPA for the five largest lipoprotein subclasses (Figure 2). For those children who recorded MVPA levels at or below the sample mean, there was a pronounced inverse trend between aerobic fitness and subclass particle numbers for the VLDL subclasses (except VLDL S), whereas the trends appear negligible for those children active above the mean level (Table 3). For example, the trends for VLDL L1 subclass were: –0.237 (–0.347, –0.127) at mean – 1 SD MVPA; –0.141 (–0.228, –0.055) at mean MVPA; and –0.045 (–0.140, 0.049) at mean + 1 SD MVPA. These inverse trend lines are increasingly divergent towards the lower end of the aerobic fitness distribution from the mean. For the other subclasses, trends at the three levels of daily MVPA did not differ greatly.

3.4. Pairwise contrasts

We found marked differences in predicted values for particle numbers of the three largest lipoprotein subclasses when contrasting two levels of daily MVPA (± 1 SD) at a level of aerobic fitness 1 SD below the mean (Table 4). For these subclasses, predicted particle numbers were higher for the lower level of MVPA (e.g., 0.41 SD units higher at a mean – 1 SD level of MVPA; p <0.001 for the VLDL L1 subclass). The estimates for the predicted values of the VLDL L3, and to a lesser extent VLDL M, subclasses were suggestive of a difference between the two levels of MVPA, but the test statistic for the contrast was above the alpha threshold (e.g., 0.26 SD units higher at a mean – 1 SD level of MVPA; p = 0.015 for the VLDL L3 subclass).

3.5. Waist circumference (strongly influenced by abdominal adiposity)

Having included waist circumference as an additional covariate in our linear models, aerobic fitness was not associated with any lipoprotein variable (p ≥0.09 for each). Given the lack of main effects, proceeding to interaction analysis was not justified (Supplementary Material Table 4).

4. Discussion

In our analysis, MVPA moderated the prospective associations between aerobic fitness and the particle numbers of certain lipoprotein subclasses. There were pronounced inverse trends for the larger ApoB-containing subclasses in children active at or below mean daily MVPA, but not at high daily MVPA. These trends were increasingly divergent at lower levels of aerobic fitness and the predicted particle numbers were markedly lower at higher levels of daily MVPA. However, the associations were greatly diminished once we included waist

circumference as a covariate in our models and suggests a strong influence of adiposity. There was limited evidence of associations with HDL particle numbers.

The association magnitudes were stronger with the triglyceride-rich VLDL lipoproteins. Though other studies have reported inverse associations between aerobic fitness and these lipoprotein subclasses, they did not investigate interaction with or effect modification by PA.[15,35] It is unclear how increased fitness might influence the production or clearance of lipoprotein particles independent of PA. In our results, trends between aerobic fitness and particle numbers of the triglyceride-rich lipoproteins were negligible at higher daily MVPA levels, which indicates that MVPA potentially drives the associations attributed to aerobic fitness. However, existing research has shown that greater adiposity is causally associated with increased concentrations of ApoB-containing lipoprotein subclasses in young adults, and with lower levels of daily total PA and MVPA in children.[36,37] Adjusting for waist circumference in our analysis strongly attenuated associations and it is likely that adiposity may confound the relationship between aerobic fitness and lipoprotein subclass particle numbers and also explain the reported effect modification at high levels of daily MVPA. The apparent lack of association between aerobic fitness and the HDL subclasses was unexpected given previously reported associations with HDL cholesterol concentration in similarly-aged children.[7] Perhaps the substantial structural, compositional, and functional heterogeneity that exists among these particles, or any potential influence of aerobic fitness, is not well-characterised using classification by particle size.[38] This finding could also be due to absence of meaningful variation of these particles in our cohort of healthy children.

We are aware of one prospective study that examined whether aerobic fitness moderated the beneficial associations between PA and cardiometabolic outcomes in children.[8] The authors found that for those children whose fitness level was below the cohort median, both MVPA and vigorous-intensity physical activity (VPA) predicted lower homeostatic model assessment of insulin resistance (HOMA-IR) and clustered cardiometabolic risk scores independent of adiposity. They concluded the importance of increasing daily MVPA, especially in the least fit children. Though we examined MVPA as the moderator as opposed to aerobic fitness, our results are similarly suggestive of a benefit of higher MVPA in less-fit children. The authors reported an association between MVPA and triglycerides concentration, which was attenuated once they included waist circumference as a covariate. The attenuation of associations with the larger ApoB-containing lipoproteins in our analysis was not surprising given that they are composed predominantly of triglycerides and that body weight is strongly associated with triglycerides concentration in children.[39] Since running performance tests of aerobic fitness are adversely influenced by body weight, and hence not a true reflection of peak oxygen uptake, the magnitude of attenuation of main effects by waist circumference is probably inflated in our study.[40]

4.1. Strengths and limitations

The use of a targeted metabolomics platform enabled us to investigate associations of aerobic fitness with lipoprotein particle numbers and whether these associations differed by subclass, which provided novel and more detailed information than the standard lipid profile. We had a high degree of compliance with our baseline measurements—demonstrated by the majority of children having at least four days of valid accelerometer data—and with the serum samples

drawn at both time points. We were therefore able to examine the temporal sequence of associations in our cohort and adjust for a number of well-recognised confounders. Still, given that our study is observational we cannot exclude the possibility that the reported associations are biased due to unmeasured confounding. For example, we lacked information on dietary intake which undeniably affects lipoprotein metabolism. The use of fasted blood samples cannot capture the influence of food intake and will have excluded a potential source of variability in our outcome measures.[41] Notwithstanding the influence of anthropometric and psychological factors on performance tests, the Andersen test has been reported as being both a valid and reliable tool for assessing aerobic fitness in school-aged children at the group level.[21] Another strength was the use of accelerometers to measure PA. Device-based measures typically exhibit lower variability than self-report methods when compared to doubly labelled water, and are less prone to the subjective biases of participants.[42,43] Though the prevalence of overweight and obesity was similar to that of children in other European nations, our cohort were atypical in that they were highly active.[44,45] Therefore, our use of sample-estimated MVPA levels in our analysis limits the generalisability of our results to other populations. This is especially true given the effect modification observed in our results occurred at a level of MVPA far higher than attained by most children. We suggest studies attempt to replicate and validate our findings in larger, more diverse populations with greater metabolic variability, over longer periods of time.

4.2. Conclusion

We provide evidence that MVPA moderates the associations between aerobic fitness and particle numbers of triglyceride-rich lipoprotein subclasses, and that there is effect modification at a higher level of MVPA. This effect is more pronounced at fitness levels towards the lower end of the distribution and indicates that encouraging higher levels of MVPA in the least-fit may be beneficial for lipoprotein metabolism. Still, adiposity has a strong influence on these associations and is likely an important modifiable risk factor. Given that the causal effect of ApoB-containing lipoproteins is determined by both the magnitude and cumulative exposure to them across the life course, primordial prevention of elevated particle numbers at a young age through lifestyle interventions could be an effective strategy to reduce CVD risk in later life.[46,47] However, to design efficacious population interventions requires better mechanistic understanding of how behaviours and physiological state influence metabolism. To that end it is essential to elucidate the causal paths between aerobic fitness, PA, and adiposity, whilst bearing in mind the broader social, environmental and political determinants of health.

References

1 Ross R, Blair SN, Arena R, *et al.* Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation* 2016; **134**: e653–99.

2 Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep* 1985; **100**: 126–31.

3 Kraus WE, Powell KE, Haskell WL, *et al.* Physical Activity, All-Cause and Cardiovascular Mortality, and Cardiovascular Disease. *Med Sci Sports Exerc* 2019; **51**: 1270–1281.

4 Clausen JSR, Marott JL, Holtermann A, Gyntelberg F, Jensen MT. Midlife Cardiorespiratory Fitness and the Long-Term Risk of Mortality. *J Am Coll Cardiol* 2018; **72**: 987.

5 Anderssen SA, Cooper AR, Riddoch C, *et al.* Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 526–31.

6 Ekelund U, Luan J, Sherar LB, *et al.* Moderate to Vigorous Physical Activity and Sedentary Time and Cardiometabolic Risk Factors in Children and Adolescents. *JAMA* 2012; **307**: 704–12.

7 Ekelund U, Anderssen SA, Froberg K, *et al.* Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. *Diabetologia* 2007; **50**: 1832–40.

8 Skrede T, Aadland E, Andersen LB, *et al.* Does cardiorespiratory fitness moderate the prospective association between physical activity and cardiometabolic risk factors in children? *Int J Obes* 2018; **42**: 1029–38.

9 Richardson TG, Sanderson E, Palmer TM, *et al.* Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. *PLOS Med* 2020; **17**: e1003062.

10 Ala-Korpela M. The culprit is the carrier, not the loads: cholesterol, triglycerides and apolipoprotein B in atherosclerosis and coronary heart disease. *Int J Epidemiol* 2019; **48**: 1389–92.

11 Ference BA, Kastelein JJP, Ray KK, *et al.* Association of Triglyceride-Lowering LPL Variants and LDL-C–Lowering LDLR Variants With Risk of Coronary Heart Disease. *JAMA* 2019; **321**: 364–73.

12 Borén J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidol* 2016; **27**: 473–483.

13 Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ Cardiovasc Genet* 2015; **8**: 192–206.

14 Jones PR, Rajalahti T, Resaland GK, *et al.* Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis* 2019; **288**: 186–93.

15 Kujala UM, Vaara JP, Kainulainen H, Vasankari T, Vaara E, Kyröläinen H. Associations of Aerobic Fitness and Maximal Muscular Strength With Metabolites in Young Men. *JAMA Netw Open* 2019; **2**: e198265–e198265.
16 Bell JA, Hamer M, Richmond RC, Timpson NJ, Carslake D, Smith GD. Associations of device-measured physical activity across adolescence with metabolic traits: Prospective cohort study. *PLOS Med* 2018; **15**: e1002649.
17 Resaland GK, Aadland E, Moe VF, *et al.* Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. *Prev Med* 2016; **91**: 322–8.
18 Resaland GK, Moe VF, Aadland E, *et al.* Active Smarter Kids (ASK): Rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on children's academic performance and risk factors for non-communicable diseases. *BMC Public Health* 2015; **15**: 709.
19 World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* 2013; **310**: 2191–4.
20 Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. *J Sports Med Phys Fitness* 2008; **48**: 434–7.
21 Aadland E, Terum T, Mamen A, Andersen LB, Resaland GK. The Andersen Aerobic Fitness Test: Reliability and Validity in 10-Year-Old Children. *PLOS ONE* 2014; **9**: e110492.
22 Esliger DW, Copeland JL, Barnes JD, Tremblay MS. Standardizing and Optimizing the Use of Accelerometer Data for Free-Living Physical Activity Monitoring. *J Phys Act Health* 2005; **2**: 366–83.
23 Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of Accelerometer Cut Points for Predicting Activity Intensity in Youth: *Med Sci Sports Exerc* 2011; **43**: 1360–8.
24 Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. *J Sports Sci* 2008; **26**: 1557–65.
25 Okazaki M. Identification of Unique Lipoprotein Subclasses for Visceral Obesity by Component Analysis of Cholesterol Profile in High-Performance Liquid Chromatography. *Arterioscler Thromb Vasc Biol* 2005; **25**: 578–84.
26 Kvalheim OM, Arneberg R, Grung B, Rajalahti T. Determination of optimum number of components in partial least squares regression from distributions of the root-mean-squared error obtained by Monte Carlo resampling. *J Chemom* 2018; **32**: e2993.
27 Lin C, Rajalahti T, Mjøs SA, Kvalheim OM. Predictive associations between serum fatty acids and lipoproteins in healthy non-obese Norwegians: implications for cardiovascular health. *Metabolomics* 2015; **12**: 6.
28 Okazaki M, Yamashita S. Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using "Spherical Particle Model". *J Oleo Sci* 2016; **65**: 265–82.
29 Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–94.
30 Carel J-C, Léger J. Precocious Puberty. *N Engl J Med* 2008; **358**: 12.

31 Spiller SA, Fitzsimons GJ, Lynch JG, Mcclelland GH. Spotlights, Floodlights, and the Magic Number Zero: Simple Effects Tests in Moderated Regression. *J Mark Res* 2013; **50**: 277–88.
32 Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008; **32**: 361–9.
33 Ferreira DLS, Williams DM, Kangas AJ, *et al.* Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. *PLOS Med* 2017; **14**: e1002376.
34 R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020 https://www.R-project.org/.
35 Resaland GK, Rajalahti T, Aadland E, Kvalheim OM. Strong association between cardiorespiratory fitness and serum lipoprotein subclass pattern in prepubertal healthy children. *Scand J Med Sci Sports* 2018; **28**: 220–7.
36 Würtz P, Wang Q, Kangas AJ, *et al.* Metabolic Signatures of Adiposity in Young Adults: Mendelian Randomization Analysis and Effects of Weight Change. *PLoS Med* 2014; **11**: e1001765.
37 Richmond RC, Davey Smith G, Ness AR, den Hoed M, McMahon G, Timpson NJ. Assessing Causality in the Association between Child Adiposity and Physical Activity Levels: A Mendelian Randomization Analysis. *PLoS Med* 2014; **11**: e1001618.
38 Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med* 2011; **17**: 594–603.
39 Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM. Cardiovascular disease risk in healthy children and its association with body mass index: systematic review and meta-analysis. *BMJ* 2012; **345**: e4759.
40 Armstrong N, Welsman J. Youth cardiorespiratory fitness: evidence, myths and misconceptions. *Bull World Health Organ* 2019; **97**: 777–82.
41 Nordestgaard BG. A Test in Context: Lipid Profile, Fasting Versus Nonfasting. *J Am Coll Cardiol* 2017; **70**: 1637–46.
42 Adams SA, Matthews CE, Ebbeling CB, *et al.* The Effect of Social Desirability and Social Approval on Self-Reports of Physical Activity. *Am J Epidemiol* 2005; **161**: 389–98.
43 Dowd KP, Szeklicki R, Minetto MA, *et al.* A systematic literature review of reviews on techniques for physical activity measurement in adults: a DEDIPAC study. *Int J Behav Nutr Phys Act* 2018; **15**: 15.
44 WHO Regional Office for Europe. WHO European Childhood Obesity Surveillance Initiative: overweight and obesity among 6–9-year-old children. Report of the third round of data collection 2012–2013. Copenhagen: World Health Organization, 2018.
45 Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1·6 million participants. *Lancet Child Adolesc Health* 2020; **4**: 23–35.
46 Ference BA, Yoo W, Alesh I, *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* 2012; **60**: 2631–9.

47 Ference BA, Graham I, Tokgozoglu L, Catapano AL. Impact of Lipids on Cardiovascular Health: JACC Health Promotion Series. *J Am Coll Cardiol* 2018; **72**: 1141–56.

Conflict of interest
The Authors declare that there is no conflict of interest.

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Authors' contributions
PRJ, GKR, EA, JS-J, SAA, TFB, TR, OMK, and UE contributed to the conception and design of the work. TR, GKR, EA, TFB, and TA contributed to data acquisition. PRJ, TR, TA, and OMK contributed to data analysis. PRJ, 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.

Acknowledgements
We are grateful to the children, parents and guardians, teachers, and principals from the participating elementary schools for their time, effort and cooperation. We thank our colleagues and students at the Western Norway University of Applied Sciences (formerly Sogn og Fjordane University College) for their assistance during the data collection. We also thank the Førde Central Hospital staff who coordinated the collection of blood samples. The NMR experiments were performed at the MR Core Facility, Norwegian University of Science and Technology (NTNU).

Figure legends

Figure 1. Flow of participants through the study indicating number of children that had valid data available

The final analytical sample included those children that had valid data for all baseline variables and blood samples at follow-up.

Figure 2. Interaction plots of aerobic fitness and lipoprotein subclass particle numbers at three levels of MVPA (*n* = 773)

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; SD = standard deviation; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Table 1. Characteristics of children included in the analytical sample (n = 773)

Characteristic	*n* (%)	Mean (SD)
Age (years)		10.0 (0.3)
Sex		
Girls	388 (50.2)	
Boys	385 (49.8)	
Anthropometry		
Height (m)		143.1 (6.7)
Weight (kg)		37.2 (8.0)
BMI ($kg \cdot m^{-2}$)		18 (2.9)
≥25	176 (22.8)	
≥30	35 (4.5)	
Waist circumference (cm)		62 (7.4)
Parents' education		
Upper secondary school	250 (32.3)	
<4 years college/university	234 (30.3)	
≥4 years college/university	289 (37.4)	
Tanner stage		
Stage 1	428 (55.4)	
Stage 2	297 (38.4)	
Stage ≥3	48 (6.2)	
Aerobic fitness		
Andersen test (m)		899.8 (102.2)
Physical activity		
MVPA ($min \cdot d^{-1}$)		76.9 (26.6)
Lipid profile (baseline)		
TC ($mmol \cdot L^{-1}$)		4.5 (0.7)
LDL-C ($mmol \cdot L^{-1}$)		2.5 (0.6)
HDL-C ($mmol \cdot L^{-1}$)		1.6 (0.3)
TG ($mmol \cdot L^{-1}$)[a]		0.7 [0.5, 0.9]
Lipid profile (follow-up)		
TC ($mmol \cdot L^{-1}$)		4.5 (0.6)
LDL-C ($mmol \cdot L^{-1}$)		2.6 (0.6)
HDL-C ($mmol \cdot L^{-1}$)		1.6 (0.3)

TG (mmol·L^{-1})[a]	0.7 [0.5, 0.9]

[a]Median [IQR].

Abbreviations: BMI = body mass index; HDL-C = high-density lipoprotein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; MVPA = moderate- to vigorous-intensity physical activity; SD = standard deviation; TC = total cholesterol; TG = triglycerides.

Table 2. Prospective associations between aerobic fitness and lipoprotein subclass particle numbers

Measure[a]	Term	Coefficient	Lower CI	Upper CI	*p* value
CM PN	Main effect	–0.134	–0.225	–0.042	0.005
	Fitness x MVPA	0.098	0.043	0.153	0.001
VLDL L1 PN	Main effect	–0.141	–0.229	–0.053	0.002
	Fitness x MVPA	0.096	0.039	0.152	0.001
VLDL L2 PN	Main effect	–0.143	–0.229	–0.056	0.002
	Fitness x MVPA	0.094	0.035	0.153	0.002
VLDL L3 PN	Main effect	–0.121	–0.195	–0.047	0.002
	Fitness x MVPA	0.057	0.003	0.110	0.037
VLDL M PN	Main effect	–0.113	–0.179	–0.047	0.001
	Fitness x MVPA	0.052	0.004	0.100	0.035
VLDL S PN	Main effect	–0.048	–0.127	0.030	0.224
	Fitness x MVPA	0.014	–0.035	0.063	0.558
LDL L PN	Main effect	–0.020	–0.087	0.047	0.549
	Fitness x MVPA	–0.010	–0.060	0.040	0.687
LDL M PN	Main effect	–0.019	–0.073	0.034	0.474
	Fitness x MVPA	–0.017	–0.062	0.028	0.455
LDL S PN	Main effect	–0.029	–0.082	0.025	0.288
	Fitness x MVPA	–0.015	–0.057	0.027	0.480
LDL VS PN	Main effect	–0.033	–0.087	0.021	0.220
	Fitness x MVPA	–0.016	–0.062	0.030	0.486
HDL VL PN	Main effect	0.004	–0.047	0.055	0.887
	Fitness x MVPA	–0.015	–0.062	0.032	0.515
HDL L PN	Main effect	0.002	–0.047	0.050	0.951
	Fitness x MVPA	–0.016	–0.062	0.030	0.484
HDL M PN	Main effect	0.016	–0.043	0.075	0.581
	Fitness x MVPA	0.000	–0.065	0.065	0.999
HDL S PN	Main effect	–0.042	–0.113	0.030	0.251
	Fitness x MVPA	0.047	–0.010	0.104	0.108
HDL VS PN	Main effect	–0.054	–0.122	0.013	0.113
	Fitness x MVPA	0.026	–0.031	0.083	0.370

Adjusted for baseline age, daily MVPA, parents' education, sex, sexual maturity, and subclass particle number. An interaction term between aerobic fitness and MVPA was included. Cluster-robust standard errors were calculated, clustered on the school variable.

[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.

Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Table 3. Simple slopes (trends) between aerobic fitness and lipoprotein subclass particle numbers

Measure[a]	MVPA level	Trend	Lower CI	Upper CI
CM PN	Mean – 1 SD	–0.232	–0.348	–0.116
	Mean	–0.134	–0.224	–0.044
	Mean + 1 SD	–0.036	–0.128	0.056
VLDL L1 PN	Mean – 1 SD	–0.237	–0.347	–0.127
	Mean	–0.141	–0.228	–0.055
	Mean + 1 SD	–0.045	–0.140	0.049
VLDL L2 PN	Mean – 1 SD	–0.237	–0.344	–0.129
	Mean	–0.143	–0.228	–0.058
	Mean + 1 SD	–0.049	–0.146	0.049
VLDL L3 PN	Mean – 1 SD	–0.177	–0.268	–0.087
	Mean	–0.121	–0.194	–0.048
	Mean + 1 SD	–0.064	–0.153	0.024
VLDL M PN	Mean – 1 SD	–0.165	–0.239	–0.091
	Mean	–0.113	–0.177	–0.049
	Mean + 1 SD	–0.061	–0.146	0.023
VLDL S PN	Mean – 1 SD	–0.063	–0.171	0.045
	Mean	–0.048	–0.125	0.029
	Mean + 1 SD	–0.034	–0.103	0.035
LDL L PN	Mean – 1 SD	–0.010	–0.112	0.092
	Mean	–0.020	–0.086	0.045
	Mean + 1 SD	–0.030	–0.085	0.024
LDL M PN	Mean – 1 SD	–0.002	–0.085	0.081
	Mean	–0.019	–0.072	0.033
	Mean + 1 SD	–0.036	–0.086	0.014
LDL S PN	Mean – 1 SD	–0.014	–0.092	0.064
	Mean	–0.029	–0.081	0.024
	Mean + 1 SD	–0.044	–0.097	0.010
LDL VS PN	Mean – 1 SD	–0.017	–0.100	0.065
	Mean	–0.033	–0.086	0.019
	Mean + 1 SD	–0.049	–0.102	0.003
HDL VL PN	Mean – 1 SD	0.019	–0.045	0.083
	Mean	0.004	–0.046	0.054
	Mean + 1 SD	–0.012	–0.083	0.060
HDL L PN	Mean – 1 SD	0.018	–0.040	0.075
	Mean	0.002	–0.046	0.049
	Mean + 1 SD	–0.015	–0.087	0.058
HDL M PN	Mean – 1 SD	0.016	–0.066	0.098
	Mean	0.016	–0.041	0.074
	Mean + 1 SD	0.016	–0.074	0.107

	Mean – 1 SD	–0.088	–0.194	0.018
HDL S PN	Mean	–0.042	–0.112	0.029
	Mean + 1 SD	0.005	–0.066	0.076
	Mean – 1 SD	–0.080	–0.181	0.021
HDL VS PN	Mean	–0.054	–0.120	0.012
	Mean + 1 SD	–0.029	–0.098	0.040

[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Table 4. Pairwise contrasts of predicted mean values of lipoprotein subclass particle numbers at *Mean ± 1 SD* aerobic fitness

Measure[a]	MVPA level	Estimate	Lower CI	Upper CI	Contrast	*p* value
CM PN	Mean – 1 SD	0.321	0.135	0.506	0.434	<0.001
	Mean + 1 SD	–0.114	–0.285	0.057		
VLDL L1 PN	Mean – 1 SD	0.333	0.145	0.521	0.408	<0.001
	Mean + 1 SD	–0.075	–0.240	0.090		
VLDL L2 PN	Mean – 1 SD	0.342	0.152	0.533	0.407	0.001
	Mean + 1 SD	–0.064	–0.236	0.108		
VLDL L3 PN	Mean – 1 SD	0.251	0.093	0.408	0.257	0.015
	Mean + 1 SD	–0.006	–0.168	0.155		
VLDL M PN	Mean – 1 SD	0.213	0.065	0.361	0.202	0.051
	Mean + 1 SD	0.011	–0.140	0.162		
VLDL S PN	Mean – 1 SD	0.013	–0.111	0.138	–0.002	0.977
	Mean + 1 SD	0.016	–0.133	0.164		
LDL L PN	Mean – 1 SD	–0.029	–0.147	0.090	–0.030	0.649
	Mean + 1 SD	0.001	–0.112	0.115		
LDL M PN	Mean – 1 SD	–0.002	–0.127	0.122	0.000	0.997
	Mean + 1 SD	–0.003	–0.109	0.103		
LDL S PN	Mean – 1 SD	0.031	–0.091	0.152	0.035	0.659
	Mean + 1 SD	–0.005	–0.123	0.113		
LDL VS PN	Mean – 1 SD	0.025	–0.099	0.148	0.033	0.683
	Mean + 1 SD	–0.008	–0.128	0.111		
HDL VL PN	Mean – 1 SD	–0.051	–0.144	0.041	–0.048	0.492
	Mean + 1 SD	–0.003	–0.136	0.129		
HDL L PN	Mean – 1 SD	–0.050	–0.137	0.036	–0.058	0.380
	Mean + 1 SD	0.007	–0.130	0.144		
HDL M PN	Mean – 1 SD	–0.085	–0.201	0.032	–0.071	0.431
	Mean + 1 SD	–0.014	–0.170	0.142		
HDL S PN	Mean – 1 SD	0.104	–0.043	0.251	0.115	0.191
	Mean + 1 SD	–0.012	–0.194	0.170		
HDL VS PN	Mean – 1 SD	0.127	–0.002	0.257	0.124	0.196
	Mean + 1 SD	0.003	–0.181	0.188		

[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Figure 1.

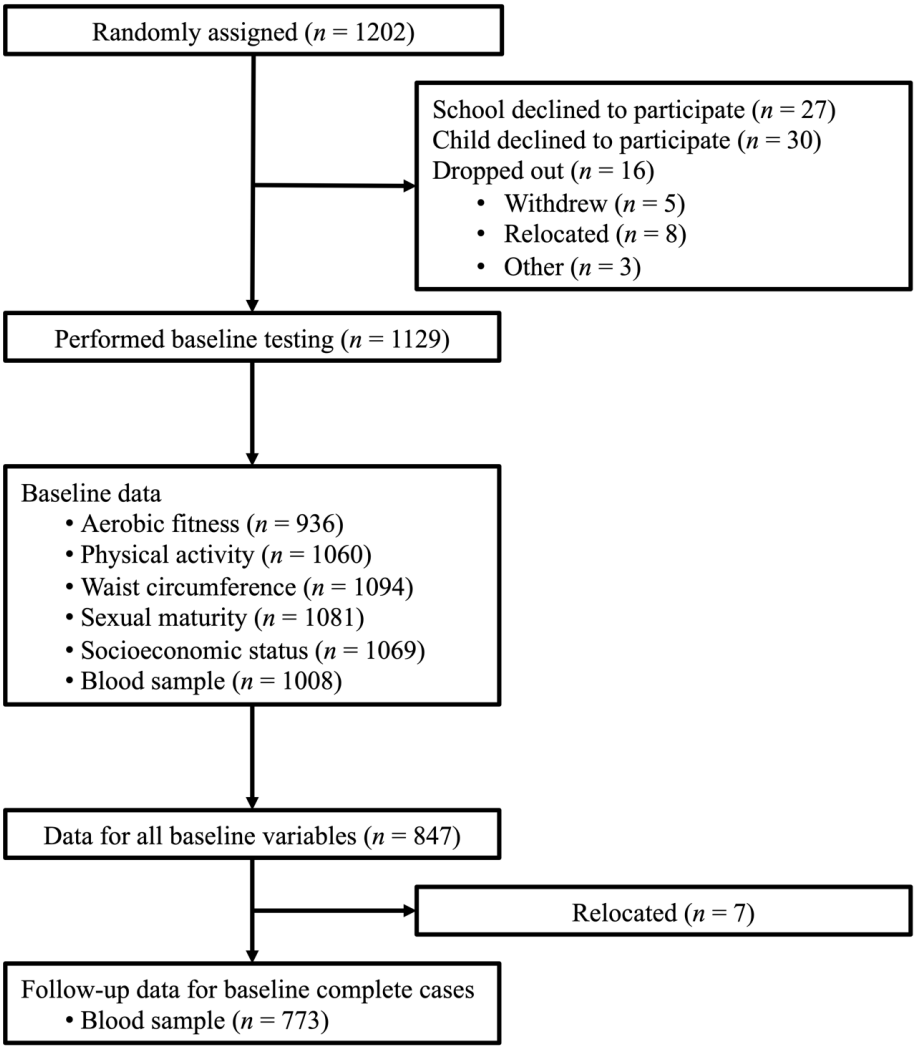
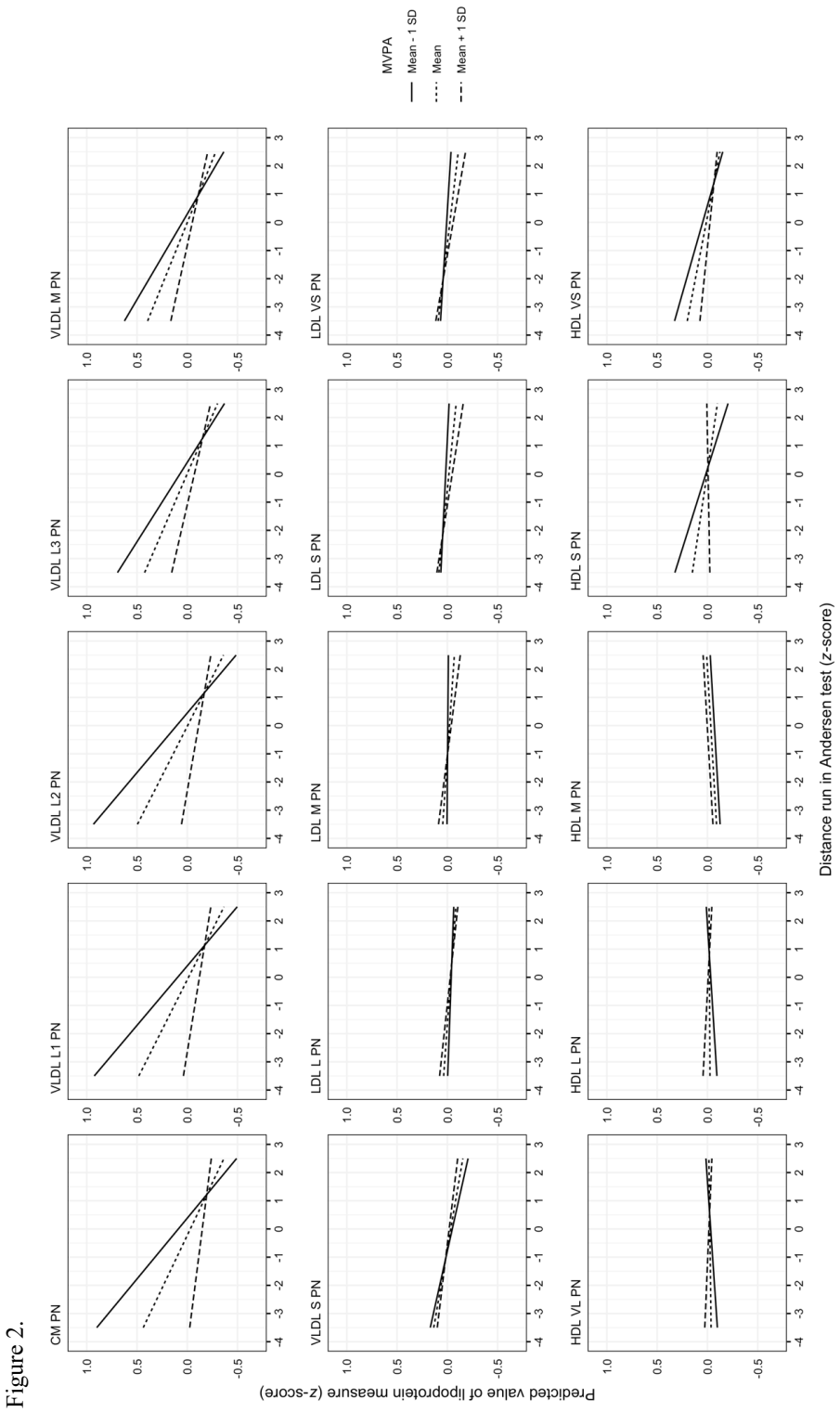

Randomly assigned (n = 1202)
School declined to participate (n = 27)
Child declined to participate (n = 30)
Dropped out (n = 16)
• Withdrew (n = 5)
• Relocated (n = 8)
• Other (n = 3)
Performed baseline testing (n = 1129)
Baseline data
• Aerobic fitness (n = 936)
• Physical activity (n = 1060)
• Waist circumference (n = 1094)
• Sexual maturity (n = 1081)
• Socioeconomic status (n = 1069)
• Blood sample (n = 1008)
Data for all baseline variables (n = 847)
Relocated (n = 7)
Follow-up data for baseline complete cases
• Blood sample (n = 773)

Figure 2.


MVPA
Mean - 1 SD
Mean
Mean + 1 SD
CM PN
VLDL L1 PN
VLDL L2 PN
VLDL L3 PN
VLDL M PN
VLDL S PN
LDL L PN
LDL M PN
LDL S PN
LDL VS PN
HDL VL PN
HDL L PN
HDL M PN
HDL S PN
HDL VS PN
Predicted value of lipoprotein measure (z-score)
Distance run in Andersen test (z-score)

Paper IV supplementary material

Supplementary methods

Blood samples

Serum was drawn from an antecubital vein and obtained according to a standard protocol consisting of the following steps: i) Blood plasma was collected in 5 ml VACUETTE Serum Gel with Activator blood collection tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria). ii) The tubes were carefully inverted five times and placed vertically for coagulation. iii) After 30 minutes, the samples were centrifuged at 2000 G for ten minutes. Serum was then visually inspected for residue and centrifugation was repeated if residue was present. iv) The tubes were kept in a refrigerator at 4°C before pipetting 0.5 ml into cryo tubes. v) The cryo tubes were then stored in a freezer at –20°C for up to 2 days before finally being stored at –80°C until analysis. The frozen serum samples were thawed at room temperature for approximately one hour. Aliquots of 120 µl were carefully mixed with equal amounts of phosphate buffer in Eppendorf tubes, and transferred to 3 mm SampleJet tubes by syringe. A fill height of 4 cm was used amounting to approximately 180 µl.

^{1}H NMR protocol

Serum spectra were recorded at 310 K, using a one-dimensional NOESY (noesygppr1d) pulse sequence. A total of 32 scans were acquired, using 96k data points and 30 ppm spectral width. The spectra were processed with 0.3 Hz line broadening, automatically phase-corrected and aligned to the lactate signal at 1.32 ppm. Spectra were normalised to an ERETIC signal, functioning as an external reference. Details of the ^{1}H NMR protocol have been described previously.[1]

References

1 Jones PR, Rajalahti T, Resaland GK, *et al.* Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis* 2019; **288**: 186–93.

Table 1. Mean and standard deviation (SD) for each lipoprotein measure in absolute concentration units

Lipoprotein variable	Baseline	Follow-up
	Mean (SD)	Mean (SD)
CM PN (nmol·L^{-1})	0.241 (0.389)	0.223 (0.304)
VLDL L1 PN (nmol·L^{-1})	1.032 (1.254)	0.962 (1.031)
VLDL L2 PN (nmol·L^{-1})	4.360 (4.614)	4.043 (3.894)
VLDL L3 PN (nmol·L^{-1})	19.326 (10.768)	18.545 (10.036)
VLDL M PN (nmol·L^{-1})	29.282 (13.649)	28.930 (12.911)
VLDL S PN (nmol·L^{-1})	45.098 (11.227)	45.767 (11.293)
LDL L PN (nmol·L^{-1})	213.306 (45.693)	217.023 (44.354)
LDL M PN (nmol·L^{-1})	469.851 (103.189)	473.121 (99.794)
LDL S PN (nmol·L^{-1})	223.074 (51.005)	222.154 (49.727)
LDL VS PN (nmol·1^{-1})	0.176 (0.037)	0.176 (0.036)
HDL VL PN (nmol·L^{-1})	0.086 (0.034)	0.090 (0.036)
HDL L PN (nmol·L^{-1})	1633.471 (725.754)	1719.370 (751.732)
HDL M PN (nmol·L^{-1})	4322.388 (602.533)	4334.860 (584.475)
HDL S PN (nmol·L^{-1})	5240.372 (539.926)	5169.407 (566.118)
HDL VS PN (nmol·L^{-1})	2678.649 (248.869)	2641.952 (252.791)

Abbreviations: CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; -C = cholesterol; -L = large; -M = medium; -PN = particle number; -S = small; -TG = triglycerides; -VL = very large; -VS = very small.

Table 2. Comparison of characteristics between those children included or not included in the analytical sample

Characteristic	Included (n = 773)		Excluded (n = 356)		Difference
	n (%)	Mean (SD)	n (%)	Mean (SD)	p value[a]
Age (years)		10.0 (0.3)	304	10.0 (0.3)	0.295
Sex					
Girls	388 (50.2)		153 (43.0)		0.024
Boys	385 (49.8)		203 (57.0)		
Anthropometry					
Height (m)		143.1 (6.7)	323	141.8 (6.9)	0.004
Weight (kg)		37.2 (8.0)	322	36.7 (8.2)	0.381
BMI ($kg \cdot m^{-2}$)		18 (2.9)	322	18.1 (3.1)	0.640
≥25	176 (22.8)		59 (18.3)		0.018
≥30	35 (4.5)		10 (3.1)		
Waist circumference (cm)		62 (7.4)	321	62 (7.6)	0.988
Parents' education[b]					
Upper secondary school	250 (32.3)		99 (33.4)		0.730
<4 years college/university	234 (30.3)		86 (29.1)		
≥4 years college/university	289 (37.4)		111 (37.5)		
Tanner stage[c]					
Stage 1	428 (55.4)		153 (49.7)		0.090
Stage 2	297 (38.4)		130 (42.2)		
Stage ≥3	48 (6.2)		25 (8.1)		
Aerobic fitness					
Andersen test (m)		899.8 (102.2)	163	882.3 (103.2)	0.048
Physical activity					
MVPA ($min \cdot d^{-1}$)		76.9 (26.6)	287	69.8 (25.0)	<0.001
Lipid profile (baseline)					
TC ($mmol \cdot L^{-1}$)		4.5 (0.7)	235	4.4 (0.6)	0.375
LDL-C ($mmol \cdot L^{-1}$)		2.5 (0.6)	235	2.5 (0.6)	0.956
HDL-C ($mmol \cdot L^{-1}$)		1.6 (0.3)	235	1.6 (0.3)	0.207

TG (mmol·L^{-1})[d]	0.7 [0.5, 0.9]	235	0.7 [0.5, 0.9]	0.713[e]
Lipid profile (follow-up)				
TC (mmol·L^{-1})	4.5 (0.6)	168	4.5 (0.6)	0.931
LDL-C (mmol·L^{-1})	2.6 (0.6)	168	2.6 (0.6)	0.860
HDL-C (mmol·L^{-1})	1.6 (0.3)	168	1.6 (0.3)	0.298
TG (mmol·L^{-1})[d]	0.7 [0.5, 0.9]	168	0.7 [0.5, 0.9]	0.481[e]

[a]Continuous characteristics compared using linear regression, categorical characteristics using binomial logistic regression
[b]For comparison, dichotomised to 'Upper secondary school' and 'Higher than upper secondary school'.
[c]For comparison, dichotomised to Tanner stage 1 and Tanner stage ≥2.
[d]Median [IQR]
[e]Comparison performed using log(TG)
Abbreviations: BMI = body mass index; HDL-C = high-density lipoprotein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; MVPA = moderate- to vigorous-intensity physical activity; SD = standard deviation; SED = sedentary time; TC = total cholesterol; TG = triglycerides.

Table 3. Prospective associations between aerobic fitness and lipoprotein subclass particle numbers

Measure[a]	Coefficient	Lower CI	Upper CI	*p* value
CM PN	–0.133	–0.226	–0.039	0.006
VLDL L1 PN	–0.140	–0.229	–0.050	0.003
VLDL L2 PN	–0.141	–0.229	–0.054	0.002
VLDL L3 PN	–0.120	–0.195	–0.045	0.002
VLDL M PN	–0.112	–0.179	–0.046	0.001
VLDL S PN	–0.048	–0.128	0.031	0.228
LDL L PN	–0.020	–0.087	0.047	0.549
LDL M PN	–0.019	–0.072	0.034	0.473
LDL S PN	–0.029	–0.082	0.025	0.288
LDL VS PN	–0.033	–0.087	0.021	0.221
HDL VL PN	0.004	–0.048	0.055	0.891
HDL L PN	0.001	–0.048	0.050	0.958
HDL M PN	0.016	–0.043	0.075	0.580
HDL S PN	–0.041	–0.116	0.033	0.270
HDL VS PN	–0.054	–0.123	0.014	0.117

Adjusted for baseline age, daily MVPA, parents' education, sex, sexual maturity, and subclass particle number. Cluster-robust standard errors were calculated, clustered on the school variable.
[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Table 4. Prospective associations between aerobic fitness and lipoprotein subclass particle numbers additionally adjusted for waist circumference

Measure[a]	Coefficient	Lower CI	Upper CI	*p* value
CM PN	–0.044	–0.139	0.052	0.363
VLDL L1 PN	–0.048	–0.135	0.039	0.271
VLDL L2 PN	–0.052	–0.134	0.030	0.213
VLDL L3 PN	–0.048	–0.119	0.022	0.175
VLDL M PN	–0.052	–0.112	0.008	0.089
VLDL S PN	–0.029	–0.111	0.053	0.483
LDL L PN	–0.012	–0.082	0.059	0.743
LDL M PN	–0.005	–0.064	0.053	0.860
LDL S PN	–0.007	–0.066	0.052	0.816
LDL VS PN	–0.015	–0.074	0.045	0.622
HDL VL PN	–0.025	–0.081	0.030	0.364
HDL L PN	–0.026	–0.080	0.027	0.331
HDL M PN	–0.026	–0.088	0.037	0.414
HDL S PN	–0.014	–0.088	0.061	0.716
HDL VS PN	–0.021	–0.090	0.047	0.534

Adjusted for baseline age, daily MVPA, parents' education, sex, sexual maturity, subclass particle number, and waist circumference. Cluster-robust standard errors were calculated, clustered on the school variable.
[a]Aerobic fitness, MVPA, and all lipoprotein measures were converted to *z*-scores prior to regression analysis.
Abbreviations: CI = confidence interval; CM = chylomicron; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVPA = moderate- to vigorous-intensity physical activity; VLDL = very low-density lipoprotein; -L = large; -M = medium; -PN = particle number; -S = small; -VL = very large; -VS = very small.

Appendix 1

Letter of approval from the Regional Committee for Medical Research Ethics

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Anette Solli Karlsen	22845522	04.03.2014	2013/1893/REK sør-øst A
			Deres dato:	Deres referanse:
			28.01.2014	

Vår referanse må oppgis ved alle henvendelser

Sigmund Anderssen
Høgskulen i Sogn og Fjordane

2013/1893 ASK - Active Smarter Kids

Forskningsansvarlig: Høgskulen i Sogn og Fjordane
Prosjektleder: Sigmund Anderssen

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 13.02.2014. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikklovens § 4.

Opprinnelig prosjektbeskrivelse
Målsettingen i dette prosjektet er å undersøke effekten av en time daglig fysisk aktivitet i skolehverdagen for elever i femte klasse.
En eventuell effekt skal måles på skoleprestasjoner i matematikk, lesing og engelsk, på kognitive prestasjoner og på helsevariabler som lipider og hjernederivert nevrotrofisk faktor (Brain Derived Neurotrophic Factor, BDNF), som påvirker hjernecellers utvikling og funksjon.
Prosjektet har et klynge randomisert design. Skolen er enheten med to grupper, en intervensjons- og en kontrollgruppe. Forsøket har en varighet på åtte måneder. I alt 1196 barn som går i femte klasse i ulike skoler i Sogn og Fjordane skal spørres om deltakelse. Halvparten av skoleklassene vil bli randomisert til intervensjonsgruppen med daglig fysisk aktivitet, mens den andre halvdelen vil komme i kontrollgruppen og får fysisk aktivitet som vanlig i skolen, dvs. to timer per uke. Den fysiske aktiviteten, som intervensjonsgruppen tilbys er variert, og etter endt forsøk, vil kontrollgruppen bli tilbudt den sammen intervensjonen dvs. når de går i 6. klasse. Med et slikt design vil alle få det samme tilbudet.
Hele utvalget vil undersøkes ved baseline og etter åtte måneder med en rekke fysiske tester, med antropometriske mål, høyde, vekt midjemål og hudtykkelse, med blodtrykk, flere kognitive tester, spørreskjema om livskvalitet, kosthold, samt vil det bli tatt blodprøver for å måle lipidmønster i blod, glukose og BDNF.
Det er utarbeidet et informasjonsskriv med samtykkeerklæring som er adressert både til foreldrene og til barna. Noen av deltakerne, dvs. barn og lærere, vil bli spurt om å delta i en kvalitativ studie, hvor intervju skal tas opp på bånd, transskriberes og analyses. I denne kvalitative delen av studien vil man også benytte seg av fotografi, dvs. man ønsker å ta bilder i de fysiske aktivitetene i prosjektet, og disse vil bli forelagt deltakerne og brukt i intervjusituasjonen.

Saksbehandling
Søknaden ble behandlet i møte 24.10.2013, og det ble fattet et utsettende vedtak. Komiteen ba om tilbakemelding på følgende punkter:

1. Datamaterialet vil bli anonymisert for forskerne i prosjektet 31.12 2016, men en navneliste vil bli

Besøksadresse: Gullhaugveien 1-3, 0484 Oslo
Telefon: 22845511
E-post: post@helseforskning.etikkom.no
Web: http://helseforskning.etikkom.no/

oppbevart hos en tredje person, dvs. hos NSD. Man opplyser også i informasjonsskrivet at man planlegger å be barna nå de er fylt 16 år om deres samtykke til å anvende data for senere forskning. Hva denne forskningen vil medføre står det ingenting om, og det går heller ikke klart fra prosjektprotokollen hva som planlegges. Prosjektbeskrivelsen omtaler ikke en slik eventuell oppfølging.
2. I informasjonsskrivet ber man om at data fra undersøkelsen kan kobles mot nasjonalt helseregister, medisinsk fødselsregister og mor/barn-registeret. Denne koblingen er ikke begrunnet noe sted, og man kan heller ikke i prosjektbeskrivelsen finne noen omtale av en slik kobling som man ber deltakerne samtykke til i informasjonsskrivet.
3. Det fins ingen opplysninger i informasjonsskrivet om den kvalitative delen av studien og heller ingen informasjon til lærerne som vil bli bedt om å delta i den delen av studien er vedlagt.
4. Prosjektledelsen har på side 8 i søknadsskjemaet diskutert ulike mulig ulemper som prosjektet kan ha på barna og argumentere for at prosjektet ikke kan ha slike ulemper som de diskuterer. En mulig ulempe er muligens uteglemt i diskusjonen og det er relatert til gruppepress. Hva med elever som ikke vil delta, for eksempel en elev i en klasse på 20 som ikke vil være med. Om hele klassen er randomisert til 1 times fysisk aktivitet hver dag, hva skjer med den ene elevens undervisningstilbud og hva kan han/hun eventuelt utsette for av mobbing/gruppepress? Det savnes en diskusjon av dette aspektet og hvordan man skal ivareta «ikke-deltakere».
5. Komiteen ber om en nærmere redegjørelse om behovet for en beredskap i forbindelse med informasjon som kan komme opp som resultat av prosjektet. Kan det tenkes uventede funn i analysene av blodprøver? Kan det tenkes svar på spørsmål i spørreskjemaet som kan tyde på det trenges en eller annen form for oppfølging?
6. Norsk versjon engelsk spørreskjema må ettersendes.

Prosjektleder har sendt tilbakemelding, denne ble mottatt 28.01.2014.

Om komiteens merknader fremkommer det av tilbakemeldingen:

1. Det kan i fremtiden være aktuelt å se på langtidseffektene av intervensjonen. Kontrolldeltakerne vil bli tilbudt samme intervensjon som studiegruppen, noe som i første omgang vil vanskeliggjøre en sammenligning mellom gruppene. Av denne grunn omfatter ikke protokollen en oppfølging på det nåværende tidspunkt. I midlertid vil en oppfølging av deltakerne i et longitudinelt design muliggjøre en evaluering av langtidseffekter, og for å sikre at man kan be barna om deltakelse i et slikt eventuelt oppfølgingsstudie ønsker man nå å legge dette inn i informasjonsskrivet. Formuleringene i informasjonsskrivet er endret slik at dersom barnet planlegges undersøkt på nytt eller dersom data vil bli benyttet etter barna er fylt 16 år, så vil man be om et nytt samtykke for dette.
2. Det skal innhentes data fra medisinsk fødselsregister og MoBa-registeret, og disse koblingene er nå spesifisert i informasjonsskrivet.
3. Det foreligger nå en beskrivelse av den kvalitative delen av prosjektet, og det er utformet separate informasjonsskriv for deltakerne i denne delen.
4. Randomiseringen til intervensjon eller kontroll vil foregå på skolenivå, og ved intervensjonsskolene vil den ekstra timen med fysisk aktivitet inngå som en ordinær del av det pedagogiske tilbudet. Det vil derfor ikke oppleves som press på enkeltelever i forhold til deltakelse i prosjektet eller ikke. For de elever som av ulike årsaker søker fritak fra fysisk aktivitet, vil skolen på ordinær måte finne andre undervisningstilbud.
5. Eventuelle funn som måtte avdekkes ved deltakelse i prosjektet vil håndteres gjennom den enkeltes skolehelsetjeneste på ordinær måte.
6. Tidligere engelske skjema foreligger nå i norsk oversettelse, dette gjelder deler av MSLQ skjemaet (management strategies, learning self-efficacy) og CCC-instrumentet (cross-curricular competencies).

Prosjektleders tilbakemelding er å anse som tilfredsstillende i forhold til komiteens merknader.

Vedtak
Komiteen godkjenner at prosjektet gjennomføres i samsvar med det som fremgår av søknaden.

Godkjenningen gjelder til 31.12.2017.

Av dokumentasjonshensyn skal opplysningene oppbevares i 5 år etter prosjektslutt. Forskningsfilen skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en datafil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato. Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren».

Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jf. helseforskningsloven § 10 tredje og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst A. Klagefristen er tre uker fra mottak av dette brevet, jf. forvaltningsloven § 29.

Med vennlig hilsen

Knut Engedal
Professor dr. med.
Leder

Anette Solli Karlsen
Komitesekretær

Kopi til: erik.kyrkjebo@hisf.no; post@hisf.no

Appendix 2

ASK study information and consent form

HØGSKULEN i SOGN OG FJORDANE

ASK active smarter kids

06. 03 2014, Sogndal

Kjære foreldre eller føresette ved 5. klassetrinn i Sogn og Fjorane, skuleåret 2014/15

Førespurnad om deltaking forskingsprosjektet «*ASK - Active Smarter Kids*»

KVA ER «ASK»?

ASK er eit stort utviklings- og forskingsprosjekt som skal undersøke korleis auka fysisk aktivitet i samspel med dei tradisjonelle faga påverkar skuleprestasjon, skuletrivsel og helse gjennom eitt skuleår (2014/15) for 5. klasseelevar.

Kva er formålet med ASK-prosjektet?

ASK-prosjektet er eit såkalla intervensjonsprosjekt som betyr at ein innfører noko nytt, for deretter å måle verknaden. For å måle verknad av ASK-modellen får halvparten av skulane intervensjonen (som er dagleg fysisk aktivitet) og den andre halvparten fortsetter som før. Skular der det er sju elevar eller meir på 5. klassetrinn i skuleåret 2014/15 vil bli inkludert i prosjektet. Skulane i kontrollgruppa 2014/15 vil få tilbod om same opplegg som prosjektgruppa, men eit år seinare (i 6. klasse, skuleåret 2015/16). Alle 26 kommunane i Sogn og Fjordane har sagt ja til deltaking i utviklings- og forskingsprosjektet ASK. Prosjektet vert gjennomført i samråd skuleregionane i Sogn og Fjordane og utdanningsaktørar i fylket. Kunnskapen som denne studien gjev vil vere viktig for å evaluere graden av kor fysisk aktive barn og unge bør vere med tanke på både læring og helse. ASK-prosjektet vil difor kunne gje samfunnet verdifull informasjon og kunnskap om organisering av skulekvardagen og metodar for førebyggande helsearbeid.

Kva inneber ASK-prosjektet for skulekvardagen til dykkar son/dotter dersom dykkar son/dotter går på ein skule som skal gjennomføre dagleg fysisk aktivitet?

Det faglege innhaldet i ASK-modellen (den daglege timen med fysisk aktivitet) blir utvikla i samarbeid mellom barneskulane i Sogn og Fjordane og HiSF, og inkluderer i løpet av ei skuleveke:

- 2 dagar x 45 minutt kroppsøving (dette gjeld alle elevar, både prosjektgruppe og kontrollgruppe)
- 1 dag x 45 minutt fysisk aktivitet (mest mogleg fysisk aktivitet på borna sine premiss)
- 3 dagar x 30 minutt «Aktiv læring» (elevane er fysisk aktive utandørs og øver på fag (t.d. mattebingo)
- 5 dagar x 5 minutt fysisk aktivitet i fag (elevane er aktive 5 minuttar i klasserommet kvar dag)
- 5 dagar x 10 minutt fysisk aktivitet i «aktiv heimelekse» (elevane er aktive 10 minutt kvar dag heime)

Den dagelge fysiske aktiviten er ikkje vurdert til å vere forbunden med risiko, og kan samanliknas med aktivitetar og metoder nytta i ein vanleg kroppsøvingstime.

Kva innber ASK-prosjektet for skulekvardagen til dykkar son/dotter dersom dykkar son/dotter ikkje går på ein skule som skal gjennomføre dagleg fysisk aktivitet?

For elevar ved skular som er kontrollgruppe, vil skuleåret gå som normalt.

Kva inneber testing i ASK-prosjektet for dykkar son/dotter?

Det vil, ved oppstart (august/sepember 2014) og avsluttning (mai/juni 2015), bli gjennomført testar for å måle verknadar av ASK. Dette er derfor ein førespurnad til dykk som er foreldre eller føresette om ditt barn kan delta på ulike testar som målar verknadar av fysisk aktivitet på skuleprestasjon, skuletrivsel og helse i ASK-prosjektet.

Testane vert gjennomført i skuletida på dei lokale skulane eller på tilrettelagde testsenter i regi av HiSF. Tilhøva som blir undersøkt er alle knytt til skuleprestasjon, skuletrivsel og folkehelse. Dette inkluderer testar for kognisjon (testar som målar t.d. hukommelse og minne), ulike spørjeskjema, test av fysisk form og fysisk aktivitetsnivå, blodtrykk, motorikk, vekt og høgde. Det vil bli teke blodprøve. Foreldre/føresette blir spurde om å fylle ut eit spørjeskjema. Dersom ein elev sitt testresultatet visar avvikande medisinske verdiar vil skulehelsetenesta informeras og informasjonen til barn/foreldre vil ved desse tilfella komme frå skulehelsetenesta. Elevane i prosjektgruppa får fritak frå undervising slik at dei kan delta i testane. Dette er testar med låg eller ingen risiko for skader, og som er gjennomført og kvalititetsikra i fleire tilsvarande studiar. I tillegg til testane over, blir fire skular valt med på ei kvalitativ undersøking, som inneber intervju og observasjon. Viss dykkar son/dotter går i ein av desse skulane, vil han/ho få utdelt eit eige informasjonsskriv og samtykkjeerklæring for denne delen av studien.

HØGSKULEN i SOGN OG FJORDANE

ASK active smarter kids

Frivillig deltaking i testar

Det er frivillig å ta del i testane i ASK-prosjektet. Ein kan trekkje seg frå heile eller delar av testane kva tid som helst og utan å oppgje grunn, og utan at det får negative konsekvensar. De kan når som helst og utan å oppgje nokon grunn trekkje samtykke. Dette vil ikkje få konsekvensar for den vidare handsaminga av dykkar barn. Dersom foreldre/føresette eller dykkar son/dotter ynskjer å trekkje seg, vil innsamla data bli sletta.

Moglege føremoner og ulemper

Under alle testane bli det lagt vekt på barnet sitt beste, og personane som er ansvarleg for testane er særs medvitne om at barn er ei sårbare gruppe. Alle moglege førehandsreglar blir tekne for å unngå eventuelle situasjonar som kan opplevast som ukomfortable for borna. Til dømes vil alle blodprøvar bli tekne i trygge lokale av røynde bioingeniørar. Me er medviten om at blodprøvetaking kan medføre psykisk påkjenningar for nokre av borna, og dersom barnet ditt ikkje ynskjer å ta blodprøven, men andre testar, er dette heilt i orden.

Kva skjer med informasjonen om dykkar barn?

Alle data som vert samla inn, både papirbasert og elektronisk, vert handsama i samsvar med krav til personvern og IKT-tryggleik nedfelt i helseforskingslova og personopplysningslova. Prøvane som ein tek og informasjonen som vert registrert om dykkar barn, skal berre nyttast i henhold til føremålet med studien. Alle skjema og data vert avidentifisert, det vil seie handsama utan namn og fødselsnummer eller andre direkte opplysningar som kan gjera at dei vert kopla til ditt barn. Identifiserbare opplysningar som knyter dykkar barn til opplysningane vert erstatta av ein kode. Lista som koplar kode og namn vert oppbevart på ein sikker måte åtskilt frå forskingsdataene, og berre prosjektleiinga har tilgang til namnelista og det er berre dei som kan finne attende til dykkar barn.

Kva skjer når prosjektet er avslutta?

Prosjektet vert avslutta 31.12.2016, men ASK ynskjer å oppbevare data for moglege framtidige oppfylgingsstudium. Datamaterialet vil 31.12.2016 bli anonymisert for forskarar i ASK, men namnelista over prosjektdeltakarar og koden som koplar dei til data vert lagra hjå ein autorisert tiltrudd tredjepart, i dette høvet Personvernombodet for forsking hjå Norsk samfunnsvitenskapelig datatjeneste. Det eksisterer i dag ikkje tilfredsstillande kunnskap vedrørande langtidsverknadar av skulebaserte fysisk aktivitetsintervensjonar, og det kan derfor bli aktuelt at dykkar barn blir spurt om å delta ved eit seinare høve. Dersom dette blir aktuelt tek me kontakt.

Resultata av prosjektet vert publisert i form av engelskspråklege artiklar i internasjonal faglitteratur. I tillegg vil resultata frå prosjektet bli formidla til det norske fagmiljøet i form av populærvitskaplege artiklar og faglege føredrag. Me skal også skrive ein rapport frå prosjektet som er retta mot deltakarane og aktørar som har vore med på å legge til rette for gjennomføringa av prosjektet. Me understrekar at opplysningar som kjem fram i publikasjonar og føredrag ikkje kan førast tilbake til einskildpersonar.

Høgskulen i Sogn og Fjordane (HiSF) er ansvarleg for forskingsprosjektet, og vil gjennomføre all testing. Prosjektleiarar er førsteamanuensis Geir K. Resaland og professor Sigmund Alfred Anderssen. Prosjektet har vore gjennom ei grundig fagleg vurdering i Norges Forskingsråd som tildelte prosjektet 17,5 millionar kronar i oktober 2012 (prosjektnr. 221047). Norges Forskingsråd vurderte ASK-prosjektet til å ha svært høg kvalitet.

Dersom de aksepterer at dykkar barn tek del i testinga i ASK-prosjektet, skriv du under samtykkjeerklæringa på neste side. Om du seier ja til å vera med no, kan du seinare trekkje attende samtykkje utan at det påverkar handsaminga di elles. Dersom du seinare ynskjer å trekkje dykkar barn eller har spørsmål til studien, kan du kontakte Geir K. Resaland.

Dersom de på noko tidspunkt har spørsmål, ta gjerne kontant på telefon eller e-post.

Venleg helsing

Førsteamanuensis Geir K. Resaland
Tlf. 57676097, Mob. 41621333
e-post gk@hisf.no

Professor Sigmund Alfred Anderssen
Tlf. Mob. 45279348
e-post s.a.anderssen@nih.no

HØGSKULEN i SOGN OG FJORDANE

ASK active smarter kids

Samtykkje til deltaking i ASK-studiet

Eg har lese informasjonsskrivet og aksepterer at mitt barn tek del i ASK-studiet

---

(Signert av foreldre til prosjektdeltakar, dato)

Eleven sitt førenamn og etternamn: (Skriv tydeleg, helst med blokkbokstavar)

……..

Foreldre/føresette sitt førenamn og etternamn: (Skriv tydeleg, helst med blokkbokstavar)

……..

Eg stadfestar at eg har gjeve informasjon om studiet

Signert, prosjektkoordinator Geir K. Resaland, dato

Appendix 3

ASK study questionnaire

T T

SPØRRESKJEMA TIL FORELDRE/FØRESATTE

ASK - Kartleggingsundersøking av fysisk aktivitet blant barn og unge i Sogn og Fjordane

Denne undersøkinga er ein del av ASK-studien. Målet med undersøkinga er å kartlegge fysisk aktivitetsnivå, haldningar til fysisk aktivitet og faktorar som assosierast med fysisk aktivitet blant barn og unge.

Informasjonen i dette spørjeskjemaet behandlast konfidensielt og er tilgjengeleg berre for dei som gjennomførar denne undersøkinga. Namneliste vil oppbevarast separat frå det øvrige datamaterialet. Skjemaet skal lesast ved hjelp av ei datamaskin. Bruk derfor svart eller blå penn ved utfylling.

Ved avkryssing: Set kryss innanfor ramma av boksen ved det svaret som passer best.

■ Om du kryssar av i feil boks, rettar du ved å fylle boksen slik.

Der du skal svare på spørsmål med tal, pass på at du skriv tydelege tal innanfor ramma av boksen. Det skal berre skrivast eit tal i kvar rute.

	7	9	,	5

Rett

Det er frivillig å delta, og du kan når som helst trekke deg fra undersøkinga. Dersom du trekk deg, vil alle opplysningar bli anonymisert. Me ber om at de svarar på spørsmåla så nøyaktig som mogleg. Dersom det er spørsmål de ikkje ynskjer å svare på kan dei hoppast over. Set berre eit kryss for kvart spørsmål.

Del A kan fyllast ut av ein av foreldra/ føresette.
Del B er retta mot barnet si mor/kvinnelege føresette og
Del C til barnet sin far/mannlege føresette.

Dersom berre ein av forelda/føresette har moglegheit for å svare på spørsmåla så ber me at det gjerast så utførleg som mogleg for begge partar.

Ved eventuelle spørsmål kan prosjektkoordinator Geir Kåre Resaland kontaktast på telefonnummer: 41 62 13 33 eller på e-post: gk@hisf.no

Ver merksam på at spørjeskjemaet har spørsmål på begge sider av arka
Vennlegast send skjemaet i den vedlagte konvolutten med din ungdom til kontaktlærar så snart du er ferdig.

T T

T T

Kva utdanning er den høgaste du har fullført? (sett eit kryss)

- ☐ Mindre enn 7 år grunnskule
- ☐ Grunnskule 7-10 år, framhaldsskule eller folkehøgskole
- ☐ Realskule, middelskule, yrkesskule, 1-2 årleg vidaregåande skole
- ☐ Artium, økonomisk gymnas, allmennfaglig retning i vidaregåande skole
- ☐ Høgskole/universitet, mindre enn 4 år
- ☐ Høgskule/universitet, 4 år eller meir

TAKK FOR AT DU HAR BESVART SPØRRESKJEMAET!

T 2T