

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

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HIGHLIGHTS

- Moderate- to vigorous-intensity physical activity is associated with a favourable lipoprotein profile in schoolchildren.
- These beneficial associations are independent of sedentary time.
- Replacing sedentary time with moderate- to vigorous-intensity physical activity is similarly beneficial.
- Sedentary time is only partly and weakly associated with an unfavourable profile.

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ABSTRACT

Background and aims: Physical activity is favourably associated with certain markers of lipid metabolism. The relationship of physical activity with lipoprotein particle profiles in children 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 min of sedentary time with 30 min 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 min of sedentary time to MVPA had broadly similar associations. Sedentary time was only partly and weakly associated with an unfavourable lipoprotein profile.

Conclusions: 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.

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1. Introduction

Insufficient levels of physical activity are associated with a number of adverse health indicators in children and youth, including cardio-metabolic 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], and with physical activity across adolescence [48]. 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 cardiovascular (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 schools (1202 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 min accumulated between 0600 and 0000 is considered a valid day. Non-wear time is defined as ≥ 20 min of zero counts [20]. Accelerometer data was collected and analysed using 10-s epochs. The accelerometer data were processed using KineSoft analytical software version 3.3.80 (KineSoft, Loughborough, United Kingdom). The physical activity outcomes are minutes/day in sedentary

time (≤ 100 counts/min), light-intensity physical activity (LPA; > 100 to < 2296 counts/min), and MVPA (≥ 2296 counts/min), classified using the Evenson cut points [21,22].

2.4. Anthropometry and maturity

Body mass (weight, 0.1 kg) 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, 0.1 cm); 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 1.0 cm, 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 600 MHz 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 120 μL were carefully mixed with equal amounts of phosphate buffer in Eppendorf tubes, and transferred to 3 mm SampleJet tubes by syringe [25]. A fill height of 4 cm was used amounting to approximately 180 μL .

One-dimensional ^1H NMR spectra were recorded at 310 K, using the *noesygppr1d* pulse sequence for water suppression. Relaxation delay and mixing time were set to 4 s and 10 ms respectively, with a low-power (25 Hz) continuous-wave pulse centred at the water frequency during both delays. A total of 32 scans were recorded, using 96 k data points

and a spectral width of 30 ppm (18 028.846 Hz). A fixed receiver gain of 90.5 was used. Line broadening of 0.3 Hz 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 15 ppm, 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 1.32 ppm.

After aligning the spectra to the lactate shifts and normalizing to the QREF-signal, we selected the shift regions describing the peaks at 1.3 ppm (approx. 900 shifts) and 0.9 ppm (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 1.3 ppm 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 parental 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

Table 1
Baseline characteristics of the included participants.

Characteristic	All	Girls	Boys	<i>p</i> ^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
Parental education, N (%)				
Upper secondary school	287 (32.6)	142 (32.9)	145 (32.3)	
< 4 years college/university	268 (30.5)	126 (29.2)	142 (31.6)	0.73
≥ 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/cm ^{2a}	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/day ^a	778 (58)	776 (56)	781 (60)	0.20
Total PA, cpm ^a	741 (288)	696 (243)	784 (320)	< 0.01
SED, min/day ^a	466 (58)	468 (57)	464 (58)	0.31
LPA, min/day ^a	233 (39)	236 (37)	231 (40)	0.08
MVPA, min/day ^a	76 (27)	69 (21)	83 (29)	< 0.01
Clinical chemistry				
TC, mmol/L ^a	4.5 (0.7)	4.5 (0.7)	4.5 (0.7)	0.63
LDL C, mmol/L ^a	2.5 (0.6)	2.5 (0.6)	2.5 (0.7)	0.54
HDL C, mmol/L ^a	1.6 (0.3)	1.6 (0.3)	1.6 (0.3)	0.02 ^d
TG, mmol/L ^b	0.7 [0.5–0.9]	0.7 [0.6–1.0]	0.6 [0.5–0.8]	< 0.01 ^e

BMI = body mass index; HDL C = high-density lipoprotein cholesterol; 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; TG = triglycerides; WC = waist circumference.

^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.

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 min. We standardised to 30 min time spent in MVPA, LPA, and sedentary time. For example, a coefficient of 0.2 for large HDL particle concentration indicates that 30 min 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-min substitution of MVPA replacing 30 min 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 Fig. 1).

3.3. Associations between intensity of physical activity and lipoprotein measures

Fig. 1 shows the associations between a 30-min 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

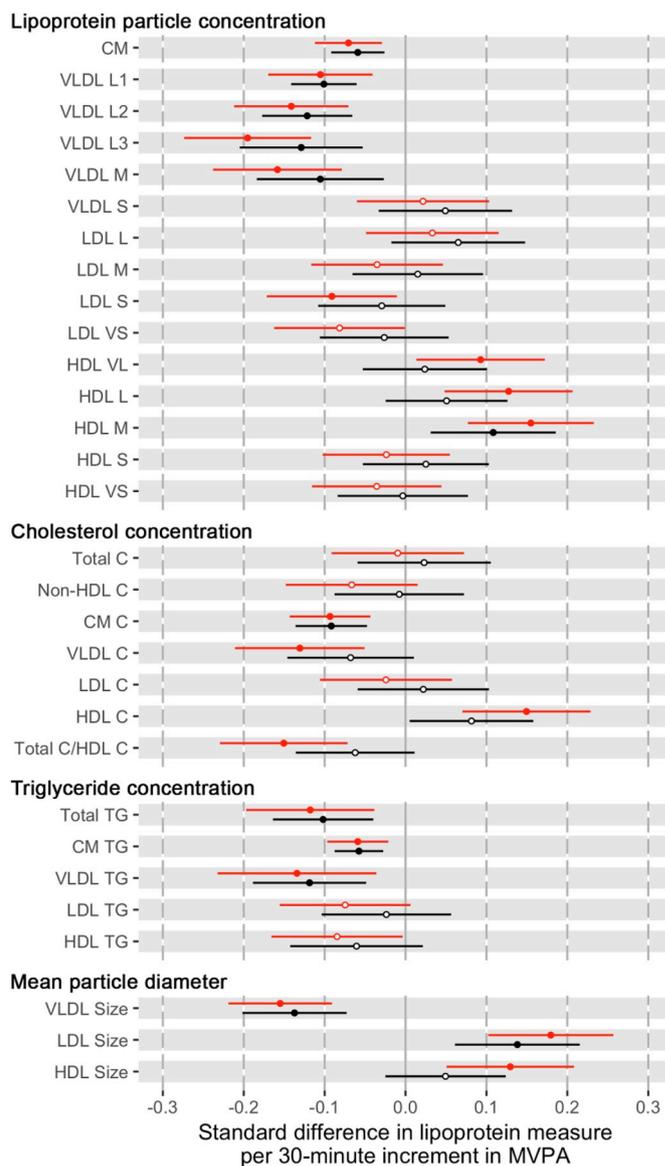


Fig. 1. Cross-sectional associations of time spent in MVPA with 30 serum lipoprotein measures (N = 880).

The associations were adjusted for monitor wear time, sex, sexual maturity and SES (red). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-min 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

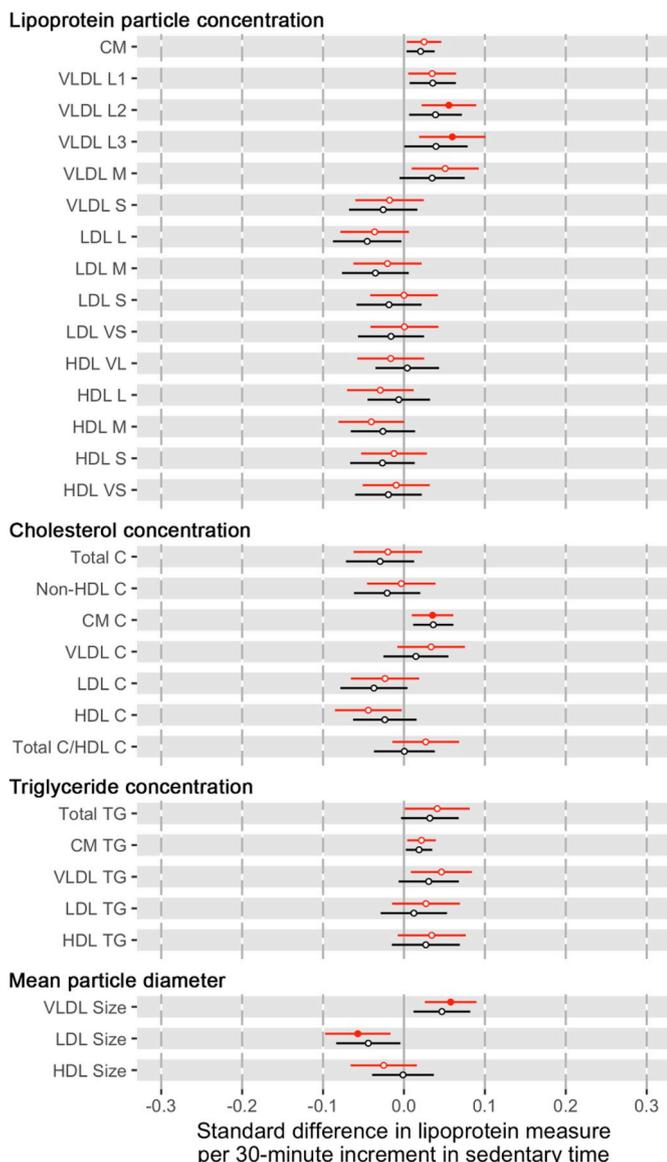


Fig. 2. Cross-sectional associations of sedentary time with 30 serum lipoprotein measures (N = 880).

The associations were adjusted for monitor wear time, sex, sexual maturity and SES (red). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-min 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

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 Fig. 2).

3.4. Associations between sedentary time and lipoprotein measures

Fig. 2 shows the associations between a 30-min 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 min sedentary time to an additional 30 min of

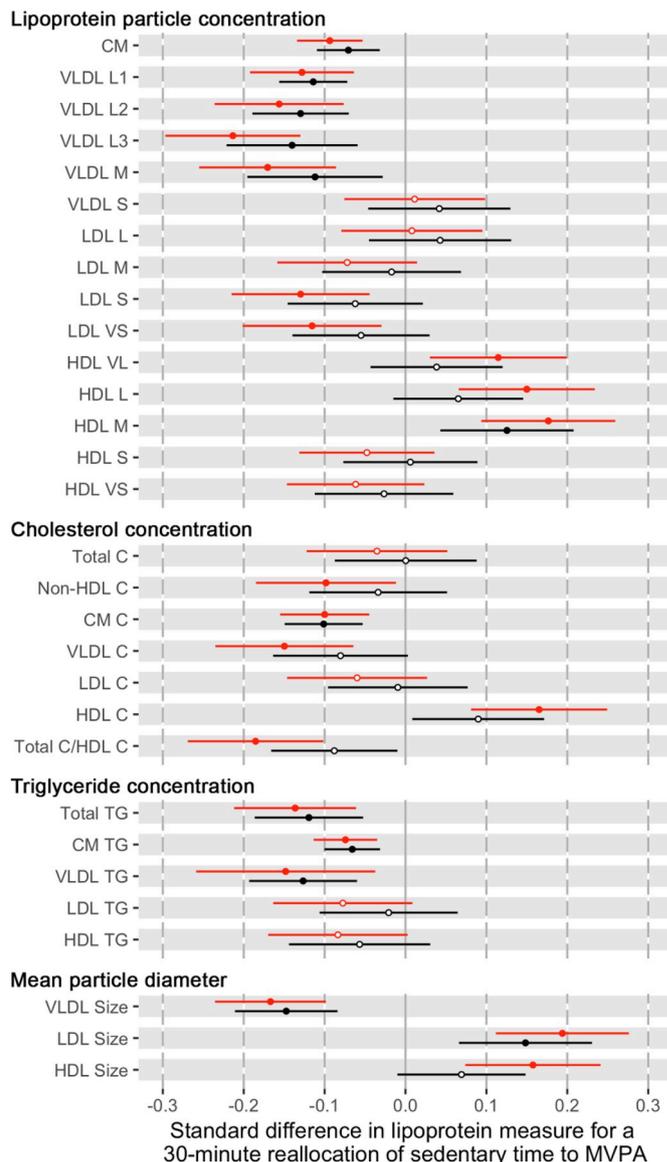


Fig. 3. Cross-sectional associations of 30 serum lipoprotein measures with an isotemporal substitution of 30 min time spent in MVPA for 30 min of sedentary time (N = 880).

The associations were adjusted for monitor wear time, sex, sexual maturity and SES (red). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure for a 30-min 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

MVPA daily produced a near identical pattern of associations with the 30 lipoprotein measures as in the single activity MVPA model (Fig. 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 associations were independent of adiposity.

3.6. Independent associations between MVPA, sedentary time and lipoprotein measures

Adjustment for sedentary time for the associations between MVPA

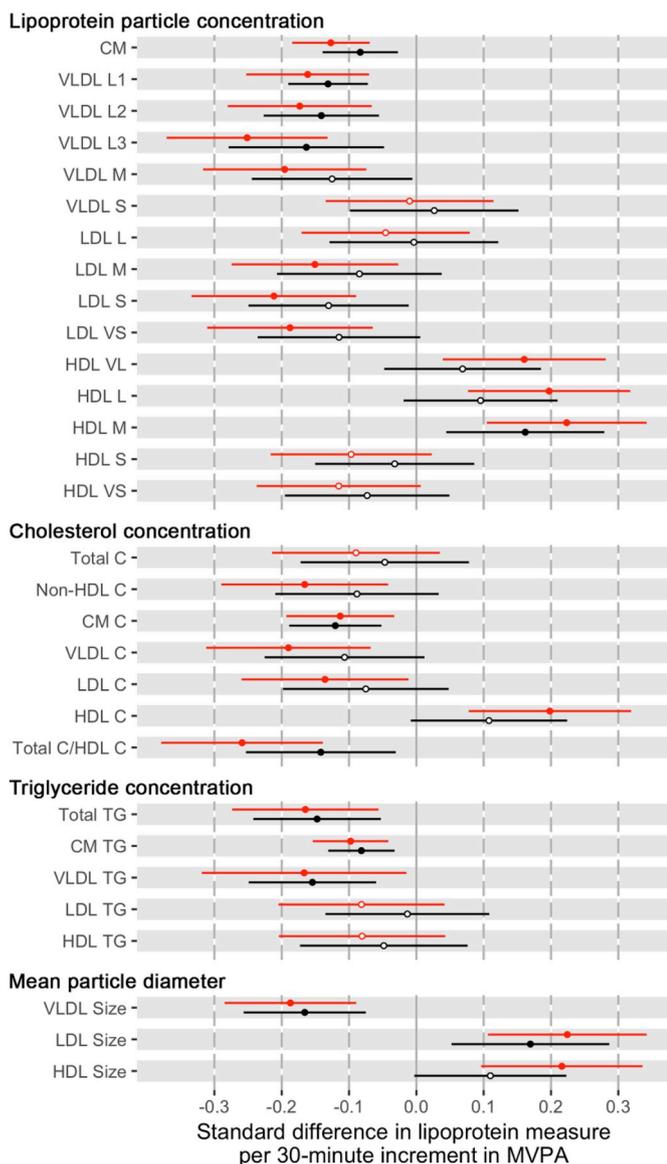


Fig. 4. Cross-sectional associations of time spent in MVPA with 30 serum lipoprotein measures adjusted for sedentary time (N = 880). The associations were adjusted for sedentary time, monitor wear time, sex, sexual maturity and SES (red). Analyses were additionally adjusted for adiposity (black). Association magnitudes are the standardised unit difference in lipoprotein measure per 30-min 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

and lipoprotein measures showed a broadly similar pattern of associations as without adjustment (Fig. 4).

A comparison of the adiposity-adjusted MVPA single activity model with and without adjustment for sedentary time is presented in Supplementary Fig. 3. Adjustment for MVPA in the model examining the associations between sedentary time and the lipoprotein measures showed no independent associations (Supplementary Fig. 4 and 5).

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. Similar results were seen in adolescents [48]. Our observations extend those previously observed in adults and adolescents 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 cutpoints [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 min 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.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.atherosclerosis.2019.05.023>.

References

- [1] L.B. Andersen, C. Riddoch, S. Kriemler, A.P. Hills, A. Hills, Physical activity and cardiovascular risk factors in children, *Br. J. Sports Med.* 45 (11) (2011 Sep) 871–876.
- [2] T. Skrede, M. Stavnsbo, E. Aadland, K.N. Aadland, S.A. Anderssen, G.K. Resaland, 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.* 105 (6) (2017 Jun) 1391–1398.
- [3] V.J. Poiras, C.E. Gray, M.M. Borghese, V. Carson, J.-P. Chaput, I. Janssen, 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.* 41 (6 Suppl 3) (2016 Jun) S197–S239.
- [4] L.B. Andersen, M. Harro, L.B. Sardinha, K. Froberg, U. Ekelund, S. Brage, et al., Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study), *Lancet* 368 (9532) (2006 Jul 22) 299–304.
- [5] Physical Activity Guidelines Advisory Committee, *Physical Activity Guidelines Advisory Committee Report*, 2008, US Department of Health and Human Services, Washington, DC, 2008 Feb.
- [6] P. Würtz, A.J. Kangas, P. Soininen, D.A. Lawlor, G. Davey Smith, M. Ala-Korpela, Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: a primer on -omic technologies, *Am. J. Epidemiol.* 186 (9) (2017 Nov 1) 1084–1096.
- [7] P. Soininen, A.J. Kangas, P. Würtz, T. Tukiainen, T. Tykkynen, R. Laatikainen, et al., High-throughput serum NMR metabolomics for cost-effective holistic studies on systemic metabolism, *Analyst* 134 (9) (2009 Sep) 1781–1785.
- [8] A.O. Akinkuolie, N.P. Paynter, L. Padmanabhan, S. Mora, High-density lipoprotein particle subclass heterogeneity and incident coronary heart disease, *Circ. Cardiovasc. Qual. Outcomes* 7 (1) (2014 Jan) 55–63.
- [9] F. Magkos, B.S. Mohammed, B. Mittendorfer, Effect of obesity on the plasma lipoprotein subclass profile in normoglycemic and normolipidemic men and women, *Int. J. Obes.* 32 (11) (2008 Nov) 1655–1664.
- [10] U.M. Kujala, V.-P. Mäkinen, I. Heinonen, P. Soininen, A.J. Kangas, T.H. Leskinen, et al., Long-term leisure-time physical activity and serum metabolome, *Circulation* 127 (3) (2013 Jan 22) 340–348.
- [11] E. Aadland, J.R. Andersen, S.A. Anderssen, O.M. Kvalheim, Physical activity versus sedentary behavior: associations with lipoprotein particle subclass concentrations in healthy adults, *PLoS One* 8 (12) (2013) 1–9.
- [12] T. Rajalahti, C. Lin, S.A. Mjøs, O.M. Kvalheim, Serum fatty acid and lipoprotein subclass concentrations and their associations in prepubertal healthy Norwegian children, *Metabolomics* 12 (5) (2016).
- [13] G.K. Resaland, T. Rajalahti, E. Aadland, O.M. Kvalheim, Strong association between cardiorespiratory fitness and serum lipoprotein subclass pattern in prepubertal healthy children, *Scand. J. Med. Sci. Sports* 28 (1) (2018) 220–227.
- [14] P. Würtz, Q. Wang, A.J. Kangas, R.C. Richmond, J. Skarp, M. Tiainen, et al., Metabolic signatures of adiposity in young adults: mendelian randomization analysis and effects of weight change, *PLoS Med.* 11 (12) (2014 Dec) e1001765.
- [15] M.P. Buman, E.A.H. Winkler, J.M. Kurka, E.B. Hekler, C.M. Baldwin, N. Owen, et al., Reallocating time to sleep, sedentary behaviors, or active behaviors: associations with cardiovascular disease risk biomarkers, NHANES 2005–2006, *Am. J. Epidemiol.* 179 (3) (2014 Feb 1) 323–334.
- [16] B.H. Hansen, S.A. Anderssen, L.B. Andersen, M. Hildebrand, E. Kolle, J. Steene-Johannessen, 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.* 48 (10) (2018 Apr 6) 2401–2412.
- [17] G.K. Resaland, V.F. Moe, E. Aadland, J. Steene-Johannessen, Ø. Glosvik, J.R. Andersen, 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* 15 (1) (2015) 709.
- [18] G.K. Resaland, E. Aadland, V.F. Moe, K.N. Aadland, T. Skrede, M. Stavnsbo, et al., Effects of physical activity on schoolchildren's academic performance: the Active Smarter Kids (ASK) cluster-randomized controlled trial, *Prev. Med.* 91 (2016 Oct) 322–328.
- [19] World Medical Association, World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects, *J. Am. Med. Assoc.* 310 (20) (2013 Nov 27) 2191–2194.
- [20] D.W. Esliger, J.L. Copeland, J.D. Barnes, M.S. Tremblay, Standardizing and optimizing the use of accelerometer data for free-living physical activity monitoring, *J. Phys. Act. Health* 2 (3) (2005) 366–383.
- [21] K.R. Evenson, D.J. Catellier, K. Gill, K.S. Ondrak, R.G. McMurray, Calibration of two objective measures of physical activity for children, *J. Sport Sci.* 26 (14) (2008 Dec) 1557–1565.
- [22] S.G. Trost, P.D. Loprinzi, R. Moore, K.A. Pfeiffer, Comparison of accelerometer cut points for predicting activity intensity in youth, *Med. Sci. Sport. Exerc.* 43 (7) (2011) 1360–1368.
- [23] C.E. Boeke, E. Oken, K.P. Kleinman, S.L. Rifas-Shiman, E.M. Taveras, M.W. Gillman, Correlations among adiposity measures in school-aged children, *BMC Pediatr.* 13 (1) (2013 Jun 24) 99.
- [24] J. Carel, J. Léger, Precocious Puberty, *N. Engl. J. Med.* 358 (22) (2008 May 29) 2366–2377.
- [25] A.C. Dona, B. Jiménez, H. Schäfer, E. Humpfer, M. Spraul, M.R. Lewis, et al., Precision high-throughput proton NMR spectroscopy of human urine, serum, and plasma for large-scale metabolic phenotyping, *Anal. Chem.* 86 (19) (2014 Oct 7) 9887–9894.
- [26] S. Akoka, L. Barantin, M. Trierweiler, Concentration measurement by proton NMR using the ERETIC method, *Anal. Chem.* 71 (13) (1999 Jul 1) 2554–2557.
- [27] G. Wider, L. Dreier, Measuring protein concentrations by NMR spectroscopy, *J. Am. Chem. Soc.* 128 (8) (2006 Mar 1) 2571–2576.
- [28] V.V. Mihaleva, D.B. van Schalkwijk, A.A. de Graaf, J. van Duynhoven, F.A. van Dorsten, J. Vervoort, et al., A systematic approach to obtain validated partial least square models for predicting lipoprotein subclasses from serum NMR spectra, *Anal. Chem.* 86 (1) (2014 Jan 7) 543–550.
- [29] S. Wold, A. Ruhe, H. Wold, W.J. Dunn III, The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverses, *SIAM J. Sci. Stat. Comput.* 5 (3) (1984 Sep) 735–743.
- [30] M. Okazaki, S. Usui, M. Ishigami, N. Sakai, T. Nakamura, Y. Matsuzawa, 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.* 25 (3) (2005 Mar) 578–584.
- [31] M. Okazaki, S. Yamashita, 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.* 65 (4) (2016) 265–282.
- [32] O.M. Kvalheim, R. Arneberg, B. Grung, T. Rajalathi, 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.* 32 (4) (2018) e2293.
- [33] C. Lin, T. Rajalahti, S.A. Mjøs, O.M. Kvalheim, Predictive associations between serum fatty acids and lipoproteins in healthy non-obese Norwegians: implications for cardiovascular health, *Metabolomics* 12 (1) (2016) 6.
- [34] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, *Clin. Chem.* 18 (6) (1972 Jun) 499–502.
- [35] R.A. Mekary, W.C. Willett, F.B. Hu, E.L. Ding, Isotemporal substitution paradigm for physical activity epidemiology and weight change, *Am. J. Epidemiol.* 170 (4) (2009 Aug 15) 519–527.
- [36] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, *J. R. Stat. Soc. Ser. B Methodol.* 57 (1) (1995) 289–300.
- [37] A.G. LeBlanc, I. Janssen, Dose-response relationship between physical activity and dyslipidemia in youth, *Can. J. Cardiol.* 26 (6) (2010) 201–205.
- [38] U. Ekelund, J. Luan, L.B. Sherar, D.W. Esliger, P. Griew, A. Cooper, et al., Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents, *J. Am. Med. Assoc.* 307 (7) (2012 Feb 15) 704–712.
- [39] M.A. Sarzynski, J. Burton, T. Rankinen, S.N. Blair, T.S. Church, J.-P. Després, et al., The effects of exercise on the lipoprotein subclass profile: a meta-analysis of 10 interventions, *Atherosclerosis* 243 (2) (2015 Dec) 364–372.
- [40] T.A. Barnett, A.S. Kelly, D.R. Young, C.K. Perry, C.A. Pratt, N.M. Edwards, 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* 138 (2018) e142–e159.
- [41] M.V. Holmes, I.Y. Millwood, C. Kartsonaki, M.R. Hill, D.A. Bennett, R. Boxall, et al., Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke, *J. Am. Coll. Cardiol.* 71 (6) (2018 Feb 13) 620–632.
- [42] S.J. Strath, L.A. Kaminsky, B.E. Ainsworth, U. Ekelund, P.S. Freedson, R.A. Gary, et al., Guide to the assessment of physical activity: clinical and research applications: a scientific statement from the American Heart association, *Circulation* 128 (20) (2013 Nov 12) 2259–2279.
- [43] A.R. Cooper, A. Goodman, A.S. Page, L.B. Sherar, D.W. Esliger, E.M.F. van Sluijs, et al., Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD), *Int. J. Behav. Nutr. Phys. Act.* 12 (1) (2015 Sep 17) 113.
- [44] M. Oliver, G.M. Schofield, G.S. Kolt, Physical activity in preschoolers: understanding prevalence and measurement issues, *Sports Med.* 37 (12) (2007) 1045–1070.
- [45] K. Corder, U. Ekelund, R.M. Steele, N.J. Wareham, S. Brage, Assessment of physical activity in youth, *J. Appl. Physiol.* 105 (3) (2008 Sep) 977–987 1985.
- [46] E. Aadland, L.B. Andersen, T. Skrede, U. Ekelund, S.A. Anderssen, G.K. Resaland, 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* 12 (12) (2017) e0189304.
- [47] J.M. Hägggi, L.R.S. Phillips, A.V. Rowlands, Validation of the GT3X ActiGraph in children and comparison with the GT1M ActiGraph, *J. Sci. Med. Sport* 16 (1) (2013 Jan) 40–44.
- [48] J.A. Bell, M. Hamer, R.C. Richmond, N.J. Timpson, D. Carslake, G.D. Smith, Associations of device-measured physical activity across adolescence with metabolic traits: prospective cohort study, *PLoS Medicine* 15 (9) (2018) e1002649.