NORWEGIAN SCHOOL OF SPORT SCIENCES

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Physical activity measured by uni- or triaxial accelerometry

Does it matter for the association to cardiometabolic risk markers in children?

Master thesis in
Department of sports medicine
Norwegian School of Sport Sciences, 2020

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Acknowledgements

What a year it has been writing this thesis! When I sit at the oh so familiar desk 2 days before the deadline I have so much to look back upon. All the countless hours at the study hall, the late nights, the "spontaneous" pizza parties and workout/blowouts/raging of frustration when SPSS complete rejects to cooperate – I wouldn't be without it for the world! As I start to reminisce on what I have learned it dawns on me through the thick fog of caffeine addiction and a periodical sedentary lifestyle that this would have been impossible to do alone…

There is a lot of people that have played a part or contributed to making this a good year for writing a thesis. To not write acknowledgements that supersedes the actual thesis I would like to thank those most important.

First to the members of "team Scandinavia", my super and co-supervisor Jakob Tarp and Ulf Ekelund. Jakob – thank you for your feedback and for always having time for me when I "just happened to be in the hallway" by your office. I really appreciate your ability to make me reflect and come to an understanding of things in my own way and time. Without you this thesis would not be what it is today.

Ulf – first as a teacher and later supervisor. Thank you for your ways of teaching and making people interested in accelerometers and Bland & Altman plots! Your input and guidance in the writing process are much appreciated!

To the both of you - I tip my hat in gratitude! Not all hero's wear capes!

I would like to thank all the other members of staff, fellow students, friends and family who have taught, challenged, discussed, supported and encouraged me throughout the last year – you know who you are!

In conclusion I would summarize this year with the quote:

"Ancora imparo" – Im still learning – Michelangelo

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Oslo - June - 2020

Abbreviations

BMI – Body-mass index

CPM – Count per minute

CRF – Cardiorespiratory fitness

DBP – Diastolic blood pressure

HDL – High density lipoprotein

LDL – Low density lipoprotein

LPA – Light physical activity

MET – Metabolic equivalent of task

MPA – Moderate physical activity

MVPA – Moderate-to-Vigorous physical activity

PA – Physical activity

SBP – Systolic blood pressure

SED – Sedentary time

TC – Total cholesterol

TC:HDL ratio – Total cholesterol: high density lipoprotein ratio

VPA – Vigorous physical activity

WThR – Waist-to-height ratio

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1. Introduction

Physical activity (PA) shows consistent cross-sectional associations to different cardiometabolic risk markers in children (1). However, these associations are less conclusive in prospective studies (2). Accurate assessment of PA is essential in epidemiological studies. The duration, intensity and patterns of sedentary time (SED) and PA are crucial to assessing the direction and magnitude of association to health outcomes, effects of interventions, population levels and trends, and to clarify which dimension of PA that is associated with a specific outcome (3). Accelerometry has gradually replaced questionnaires as a feasible method applicable in large population-based studies. The most frequently used accelerometer is the ActiGraph (4). Their earlier models recorded activity on a uniaxial basis, but after the introduction of the GT3X the use of triaxial accelerometry has become possible. In a triaxial output the medio-lateral, vertical, and antero-posterior axis are squared and combined into a vector magnitude. When recording human movement by accelerometry most acceleration is generated from walking behavior and the vertical displacement of the center of mass. The total vertical displacement increases with step frequency which are largely dependent on body height and lower extremity length (5). The recorded acceleration goes through filters and digital converters to create a raw acceleration signal. The amount of time spent at different intensities are determined by applying pre-established cut points. These cut points categorize the acceleration into SED, light physical activity (LPA), moderate physical activity (MPA) and vigorous physical activity (VPA) which is usually expressed in a count per time interval (e.g. counts per minute (CPM)). These different PA counts are arbitrary, differ between ages (6) and are used as an expression of PA intensities (7). In a vector magnitude the vertical axis explains the majority of CPM with up to 95% of the variance during daily routine movements (8). The difference in the final PA outputs intensity classification from the vector magnitude and vertical axis seem dependent on the type of activity and intensity of the activity being done (8). The correlation to doubly labeled water determined PA energy expenditure differs between uni- and triaxial accelerometry. Uniaxial correlation to doubly labeled water determined PA energy expenditure is low to moderate (0.37-0.56), but higher correlations are found for triaxial accelerometry (0.32-0.79) (9). This suggests that using triaxial accelerometry provides a better measure of total PA volume. Recently it has been investigated how uni- and triaxial PA are associated with cardiometabolic health in

children. The uni- and triaxial PA levels varied but were highly correlated (r=≥0.72) (10). When analyzing the medio-lateral, vertical and antero-posterior axes separately, they were all associated with cardiometabolic health, but their strongest association varied in the amount of CPM. The vertical axis showed the strongest association to cardiometabolic health at 7000-7499 CPM while the antero-posterior axis was strongest at 6000-6499 CPM and the medio-lateral axis 100-249 CPM. The vector magnitude pattern reflected the three axes combined and showed the strongest association at 9499-9999 CPM. The vector magnitude achieved a higher model fit (R^2=18.9%) than the vertical axis (R^2=17.0%). These results suggest different associations between uni- and triaxial derived PA and cardiometabolic health and a potential for future use of triaxial accelerometry (10).

Gaps in current research

The article by Aadland and colleagues broke new ground in accelerometer research by uncovering a considerable difference between uni- and triaxial accelerometry and its association to cardiometabolic risk markers. This was the first article to compare the vertical axis and vector magnitude from ActiGraph accelerometers in children. They applied a multivariate pattern analysis to their data which we don't know how compares to the traditional cut point approach (10). Their results were cross-sectional which is prone to reverse causation that are avoidable with prospective studies that can adjust for outcome baseline values and be able to account for potential benefits of altered PA levels. The uni- and triaxial difference remains unclear for the time spent in different PA intensities and the magnitude and shape of association to cardiometabolic health.

Study aim

This study will compare uni- and triaxial derived time spent sedentary, light, moderate, vigorous, and moderate-to-vigorous physical activity (MVPA). We will investigate if uni- and triaxial accelerometry has implications for the magnitude and shape of the prospective association to cardiometabolic risk markers in children. We aim to: Compare the magnitude and shape of association between SED, LPA, MPA, VPA, and MVPA from uni-and triaxial accelerometry in different epoch lengths to a composite cardiometabolic risk score in children.

Uni- and triaxial differences

When recording human movement different activities can consist of complex agile motion. The collection and storage of uni- and triaxial PA outputs for ActiGraph differs. A triaxial signal includes the medio-lateral = x, vertical = y and antero-posterior = z axis. Combining these and squaring them $\left(\sqrt{x^2 + y^2 + z^2}\right)$ gives us a vector magnitude. There has been indications that uni- and triaxial accelerometry are comparable for assessing SED, but methods and protocols varies across studies (11). The time spent in different PA intensities vary with the data processing criteria used. Comparison between studies are complicated as this would require standardization of data processing across studies. The studies comparing uni- and triaxial accelerometry have used two or more different accelerometers, which limits comparability and complicates interpretation of results. Accelerometer post-processing decisions affects the amount of time spent in each PA intensity estimate. These differences are largely dependent on post-processing decisions and lead to limited agreement between datasets. The final PA intensity estimates are considered data reduction specific (12). In the vector magnitude output the vertical axis generally explains the majority of CPM. However, this is largely dependent on what activity and the intensity of it as the uni- and triaxial difference grows as the intensity increases. This is especially evident in activities that consists of complex, rapid and agile movement (8). Triaxial accelerometry are expected to record a wider range of movement in activities compared to uniaxial accelerometry. The use of triaxial accelerometry results in significant differences for all PA intensities in adults with less SED and more time spent in LPA and MVPA registered compared to uniaxial outputs (13). The increase in MVPA leads to higher PA guideline compliance. It seems that triaxial accelerometry captures more bouted and non-bouted movement than uniaxial, most prominently non-bouted MVPA (13). Uni- and triaxial differences are smaller in laboratory settings, probably due to movement restricted activities used in calibration studies. In laboratory conditions the activities used may not include enough movement along the additional medio-lateral and antero-posterior axes, which could be expected in free-living conditions (14). Activities and movements along the medio-lateral axis or rotational movement (e.g. dancing or gymnastics) will to some degree always involve some vertical displacement (8). The difference between uni- and triaxial registered PA seem dependent on what type of activity and the intensity of it. Activities with a high triaxial signature could

possibly result in PA estimate differences large enough to affect associations to cardiometabolic risk markers.

Physical activity, sedentary time, and cardiometabolic risk markers

PA is considered a continuum of intensity consisting of SED, LPA, MPA and VPA, where MPA and VPA often are combined into MVPA (15). SED is defined as activity not surpassing an energy demand of ≤1.5 metabolic equivalent of task (MET) while in a sitting or reclining posture (16). LPA is defined as <4 MET, MPA <6 MET, VPA >6 MET and consequently MVPA >4 MET (17) when categorizing PA intensities in children. Accelerometer assessed PA, especially higher intensities, is associated with cardiometabolic risk markers (2, 18). LPA seems favorably associated with high density lipoprotein (HDL), blood pressure and adiposity (19). Additional LPA should be considered beneficial to cardiometabolic health in adjunction to fulfilling the PA recommendations if possible. LPA is more feasible, but less potent than MVPA (19). Objectively assessed MVPA is consistently associated with cardiometabolic risk markers. The strongest prospective associations is to insulin (18, 20, 21), triglycerides (2, 22, 23) and clustered risk (2). The associations is moderated by cardio respiratory fitness (CRF, 24,25) and adiposity (25-27). Furthermore, adjusting for MVPA, moderates other associations (28). Certain aspects of SED, such as TV or screen time, is associated with levels of adiposity when assessed by questionnaires (29), but may be confounded by high energy food and beverage intake (30). There is no evidence for any prospective association between SED and any cardiometabolic risk markers when assessed by accelerometry (2). In this thesis SED will not differentiate types of activities as we are not able to clarify the context of the behavior with our accelerometer data.

Physical activity registration in children

Childrens PA level has previously been assessed through direct observation in small groups and by proxy report in larger samples through different questionnaires. As accelerometers became a feasible method and gradually replaced questionnaires the PA studies among children were revolutionized. Compared to adults, children produce higher step frequencies to compensate for shorter stride length (5). Most childrens daily

PA are walking behavior just like adults, but childrens high intensity PA are thought to occur in a more sporadic and intermittent manor, either through organized sport or recreational play (31). The agreement between uni- and triaxial accelerometry varies in different activities and sports (8). The PA registration process, differences and how this relates to this thesis should be described.

The accelerometer records acceleration as an interpretation of human movement. The first ActiGraph accelerometers had all the processing software integrated in the accelerometer where the end product to the user was given as activity counts. Newer generations of ActiGraph accelerometers allow for raw data storage and activity registration for multiple weeks. A raw data signal consists of a gravitational component, movement and noise. Values are expressed in milli (10^{-3}) gravity-based units (mg) where g=9.81 m/s^2 (earth's gravity). Researchers can apply different processing criteria to the raw data where the purpose is to give an estimate of bodily movement by removing gravitational acceleration and noise from the signal (32). The signal provides information about PA duration, intensity, patterns and inactivity. This is expressed in activity counts, a unit that's arbitrary to the accelerometer make and is without any biomechanical or physiological meaning (7, 33). The counts are summed up into epochs, where the epoch length determines the time period that activity strings are aggregated and summarized. For example, a 10-s string of 2000-2500-2500-2500-2000-3000-3500-3500-4000-4500 CPM would result in 6-s of LPA, 3-s of MPA and 1-s of VPA in an 1-s epoch, whilst 5-s of LPA and MPA in a 5-s epoch (mean 2300 and 3700) and 10-s of LPA in a 10-s epoch (mean 3000) when using the vector magnitude cut-points of Romanzini (34).

From a biomechanical standpoint, most counts generated by walking behavior stems from vertical displacement from a person's center of mass, which makes stride length a key determinant of walking/running. When walking/running speed increases so does the stride length, but the frequency only to some degree (35). There is a linear increase in vertical displacement when increasing walking speeds, with a major leap when transitioning into running (5). For a given speed, children in general produce higher step frequencies than adults to compensate for a shorter stride length. This suggests that the children in our sample would produce increasingly more acceleration when walking and running compared to adults at the same speed. This pattern would become even more

distinctive if we were to compare the vector magnitude to the vertical axis. The vertical displacement and hence counts, levels off when reaching running speeds of 10-12 km/h (33). This is problematic seeing that individual gait patterns may vary greatly, and movement frequencies may therefore rise above ActiGraph filters band pass limits, which then would lead to attenuation of the acceleration frequency signal (5, 7). This does however only affect our activity registration to a lesser degree, as children of our samples age are thought to be unable to uphold such running speed for longer periods of time. It has been proposed that widening the ActiGraph band pass filter (Standard ActiGraph filter is 2.5 Hz) to 4 and up to 10 Hz may increase accuracy for capturing movement and distinguish PA intensities, especially MPA from VPA. Widening the frequency range reduces measurement bias with running activities above 10 km/h. However, even with better classification of PA intensities there were no significant increase in free-living measured VPA by widening the frequency range compared to the original ActiGraph filter (36). When using narrow filters, the filtering process might be influenced by the high step frequency seen in children, leading to more acceleration being filtered out as noise. It's been proposed that the relative contribution of noise in the signal, especially in children, leads to a higher misclassification of lower PA intensities, generally misclassifying SED as LPA (37). However, when widening the filter, it led to a substantial increase in measured acceleration at higher intensities. The frequencies were not evenly distributed, but dependent of the intensity. Widening the filters had little impact on misclassification of SED and LPA, instead there was major disagreement between MPA and VPA (37). The filters purpose is to attenuate non-human movement or eliminating low frequency movement. Acceleration in human movement is almost exclusively found between 1-10 Hz so widening filters further seems unnecessary, especially for our age group. This could be explained by childrens higher step frequency of walking/running, where each step would exert an acceleration (movement), deacceleration (gravitational component) and a ground contact phase (noise) thereby increasing the total amount of noise being filtered out related to MPA and VPA (37). Because step frequency is proportional to the internal work, whereas the vertical displacement of the center of mass is proportional to the external work the increased step frequency would potentially lead to more noise being filtered out (5).

Accelerometer processing criteria and physical activity estimates

When assessing PA by accelerometers there is multiple data processing criteria to consider. Device placement, non-wear time criteria, valid day criteria, sampling resolution, filter function, epoch length and intensity cut points all have the potential to influence the final PA output. Decisions regarding device placement, sampling resolution and the use of filter function are taken before collecting the data. Which non-wear time and valid day criteria, epoch length and intensity cut points to use for the raw acceleration data are applied afterwards.

The accelerometers placement varies mainly between hip and wrist. In adolescents there have been reports of higher compliance with wrist-worn than hip-worn accelerometers (38). However, similar wear-time compliance at the hip is seen when implementing a 24 h/day wear-time protocol. (39). Device placement results in differences in intensity classification, with higher outputs of SED and all PA intensities from wrist than at the hip. The intensity classification accuracy is generally higher for SED and LPA than >MVPA when comparing wrist-worn to hip-worn measured PA (40). The ability to discriminate between different types of SED seems to vary between laboratory/calibration settings and free-living measurements (41). When comparing non-dominant to dominant wrist and hip-worn placement, hip-worn achieved the highest ROC-AUC of >0.90 for all cut-points. Even though large studies such as U.S. National Health and Nutrition Examination Survey (NHANES) use wrist placement for increased compliance (42) and the correlation to doubly labelled water are similar (43) hip placement has historically been the favored device placement (6). This could be attributed to the postural capabilities of hip-worn to classify SED, as a person might be lying or sitting in a supine position and still register activity by moving their arms and/or upper body (43). Additionally, activity is largely dependent on movement of the center of mass (5).

Accurately assessing non-wear-time is crucial to get a precise estimate of actual wear-time. The difference between SED and time when the accelerometer is not worn, is a major problem for researchers. When analyzing accelerometer data, consecutive strings of zero acceleration are identified and excluded from the total wear-time estimate (44).

The choice of non-wear-time has the potential to influence both sample size and the amount of registered SED. Shorter non-wear-time criteria lead to higher total CPM per day by the exclusion of more time with zero recorded counts. Furthermore, the percentage portion of SED decreases and all PA increases, with the opposite effect observed with longer wear-time criteria (6). One study compared 10 different non-wear-time criteria with adjacent logbooks. Their criteria were >10, 20, 30, 45, 60, 90, 60-1, 60-2, 90-1, 90-2, where -1 or -2 allows for short 1- and 2-min breaks in a string of zero counts. While there were differences in the amount of SED, there was no significant difference in time spent in LPA, MPA, VPA or MVPA with any duration used (45). Another study compared four non-wear-time criteria (10, 20, 60 and 100 min of zero string) with different sedentary cut-points in 9- and 15-year olds. They found a significant increase in mean hours of SED per day between cut-points. However, there was no significant difference between the non-wear-time criteria within each sedentary cut point (46). Similar result were reported when upholding the non-wear-time criteria of consecutive 20 min of zero string, where the PA estimate did not differ significantly by epoch length (1, 5, 10, 15, 30 and 60-s) (47). There is no consensus regarding which non-wear-time criteria that is best, as it seems to vary with the population being studied (45, 48). Over the last decade there seems to be a trend to use shorter non-wear-time criteria. This is possibly explained by the decrease in PA with age in children and adolescents (49).

Similar to non-wear-time criteria, the valid day criteria has effect on sample sizes, as more h/day needed for a valid day, the smaller sample size and consequently the study's statistical power (48). The required overall wear-time to accurately capture individuals usual PA pattern varies. Recommendations vary by gender and age, usually between 3-10 days. There have been recommendations that at least 7 days is enough to acquire reliable PA estimates, but 3 days is the most frequently used inclusion criteria (44). The fewer amount of days needed generally lead to bigger sample sizes, where more days needed has the opposite effect. As the number of days increases so does the focus on acquiring compliance rates to upkeep the sample sizes. This could be a reason why researchers use less stringent criteria (44).

Since the release of the GT3X+ sampling between 30 and 100 Hz (in a 10 Hz interval) has been possible. The higher resolution, the more detailed data, as the resolution

determines how many times per second the acceleration-signal is recorded and stored. The chosen resolution should reflect what and who are being investigated as sampling frequency have shown to affect activity counts. Similar activity estimates are seen when using 30, 60 and 90 Hz, which is referred to as the "multiplication principle". When comparing those sampling frequencies to other frequencies it led to increased activity counts. For instance, when comparing 40 vs 30 Hz an increasingly higher total CPM are observed for different walking and running speeds. Differences are credited to manufacturers original filters which originally were developed for 30 Hz (50). In children the filter function does not alter the total CPM significantly. There have been comparisons between the ActiGraph normal filter function and the low frequency extension filter. Where the ActiGraph filters purpose is to attenuate acceleration signals outside of the normal frequency of human movement, the low frequency extension filter extends the lower end of the cut-off, therefore making the measurement more sensitive (51). The use of different filters seems to be dependent on what type of activity that is being investigated. When investigating sleep, the difference over the course of 8h with normal and low frequency extension filter resulted in 6 CPM more when the low frequency extension filter was used (51). When widening the dynamic filter (0.29-1.66 Hz) from the original ActiGraph filter to 4 and 10 Hz an increasingly higher linear increase in CPM up to running speeds of 10 km/h were observed between the filter width (37).

The short and intermittent bouts of PA typically seen in children calls for shorter epochs to accurately capture PA. For children the recommended epoch lengths is between 1 - 15-s (6). PA was previously captured using a 60-s resolution in children, due to device storage capabilities. More recently shorter epoch lengths have been deployed, typically being <15-s (44). The association between PA and cardiometabolic health are affected by epoch length, and bout duration. When using different bout durations ranging between 1-s – 60-min and analyzing them in 1, 10 and 60-s epoch length the total amount of SED, LPA and VPA varied, while overall PA, MPA and MVPA were unaffected (52). The time spent in each intensity was dependent on the epoch setting. The largest portions of MPA, VPA and MVPA was accumulated in 1 and 10-s epoch lengths and in the shortest bout durations (1 - 9-s for 1-s epoch and 10 - 39-s for 10-s epoch) (52). Cut points are reintegrated into smaller or larger epochs in many studies. Although short epochs are recommended for children few cut points are calibrated in

short epoch (e.g. 1 – 5-s). Reintegration into smaller or larger epochs is done with the assumption that this does not change PA classification. One study found the activity counts and MVPA estimates mean difference to be minimal and not significant (53). The study by Logan et al found no differences in the total mean CPM between any reintegrated epoch lengths for the vector magnitude. However, reintegrating the Romanzini cut points from 1 - 60-s (1-5-15-30-60-s) resulted in a decrease in SED, MPA, VPA and an increase in LPA (54). There are various epoch lengths used in studies on children. If the bout length of PA is shorter than the epoch length it can lead to misclassification of PA intensities (55). Studies have shown that by increasing the epoch length, SED and VPA decreases, while LPA and MPA increases (54-56). The differences in PA estimates when reintegrating epochs can be substantial, with so much as >150 and >160 min/day of SED and LPA when reintegrating from 1 to 60-s epoch. This might potentially skew associations between PA intensities and cardiometabolic risk markers as each intensity are differently associated (55). The strongest association to cardiometabolic health varies with epoch lengths, where longer epoch could lead PA estimates to have stronger associations at lower amounts of CPM (57). The accumulation of different intensities vary the most when comparing the shortest (1,2,3,5 and 10-s) to the longest (30 and 60-s) epochs commonly used (55). The shortest epochs seems to be the most comparable when reintegrated from shorter epochs in children (55, 56). This further strengthens the growing consensus that calls for shorter epoch lengths when assessing childrens PA level (58, 59).

There is no consensus about what set of cut-points to apply to accelerometer data. Cut points vary for what and who is being investigated and there is a difference between the use of vertical axis and vector magnitude cut points (60). When creating cut points, the PA intensity thresholds are calibrated using different activities representable for SED and different PA intensities. The activities are carried out while the oxygen consumption is measured by a metabolic system. Some have used whole room calorimetry, but the most common is a portable metabolic system. The protocol includes activities or behaviors that are categorized by certain MET thresholds. The most common thresholds of <1.5, >1.5, >4 and >6 MET are used to reflect SED, LPA, MPA and VPA respectfully, in children (17). There have been disagreements between the use of 3 or 4 MET as the threshold for MPA in children, but seeing how walking behavior that make up most of daily routine activity closer approximate 4 MET (17) and that

children's resting energy expenditure is greater than adults by relative weight (61), 4 MET seems most appropriate to classify MPA. The participants are instructed to do different activities, household chores or other behaviors typical to a daily life routine. Calibration protocols usually have 2-4 different activities for SED or PA intensities that are presumed representative for that specific behavior at whole. These activities seem arbitrarily chosen as there is no standardization across studies. They do however include treadmill walking at different speeds that are either voluntarily selected by the participants, researchers or extracted from the compendium of physical activities. The discrepancy between MET thresholds for MPA and individual gait pattern differences further complicates comparisons between studies using different cut points.

Cut point consideration

The cut points by Evenson and co-workers were validated and developed for the vertical axis. They used a pre-established regression equation of the VO_2 measures and the heart rate of their participants at 2,3 and 4 mph at treadmill walking/running to determine their predicted $VO_{2\text{-max}}$ and maximum heart rate. These values were used to calculate the most sensitive and specific thresholds for each cut point, were all cut points showed a good (>0.80) ROC curve (62). They categorized SED as <100 CPM, LPA >100, MPA >2296 and VPA as >4012 CPM. These cut points are frequently used in comparison to others in youth (17), which is related to their calibration protocol including activities that are less structured and more indicative to free living activity (62). The use of vector magnitude in epidemiological studies are rare, and few ActiGraph cut points exist that are eligible for our study sample of 5^{th} graders. To be able to answer our research question the cut points for vertical axis and vector magnitude need to be calibrated from the same data. There are a limited number of studies that have developed vector magnitude cut points applicable to our sample. One study validated cut-points in 5-9-year-old (mean age 7.5-y) children using a 5-s epoch setting. Their protocol consisted of different walking/running speeds (2, 4, 6 and 8 km/h) on a treadmill followed by some free-play and ball activities (63). Similarly, Hänggi and colleagues developed vector magnitude cut points in 1-s setting using children between 10-15 years old (mean age 10.8-y). Their calibration protocol consisted of activities lying down, sitting, standing, Nintendo wii boxing and different treadmill speeds. The treadmill speeds were determined in collaboration with the child to represent slow-brisk walk and slow-

medium running speeds (11). Another study used prediction equations determined by an artificial neural network model to develop cut points for the vertical axis and vector magnitude. They used a sample of 31 adolescents with a mean age of 14.7 years old for predicting combined cut points for children/youth. They calculated cut points for MPA, VPA and very vigorous PA equaling 3, 6 and 9 MET (64). The aforementioned studies share some limitations. Neither of them provides cut points for all intensities (SED, LPA, MPA, VPA). Jimmy et al and Santos-Lozano et al does not provide cut points for SED, while Hänggi et al do not separate MPA and VPA in their MVPA cut point. This would limit the comparability to other studies. The studies use a variety of different activities and different walk/running speeds. The lack of standardization of activities and speeds makes the cut points difficult to compare. These calibration protocols would have greatly benefited from considering the accelerometer movement registration when using triaxial accelerometry and the device placement. All the mentioned studies include treadmill walking/running in their calibration protocol, even though it is easy to standardize, the majority of movement stem from the vertical axis. The lack of activities that would to a greater extent include the antero-posterior and medio-lateral should be considered. The study by Hänggi included a 3 minute period of Nintendo Wii boxing which in theory could be representative of childrens video gaming, but video gaming would in most cases be carried out in a sitting or reclined position that would better represent SED than LPA or in some cases MPA. In this case boxing would be a more appropriate activity if the accelerometer were attached at the wrist rather than the hip. Furthermore, the treadmill speeds used were randomly selected in collaboration with the child. The authors themselves argues that this is a weakness as a conversion factor between the number of axes included could not be calculated due to the variation of agreement within the activities. The study by Santos-Lozano uses an artificial neural network to determine their cut points. This method is rather complicated to use and understand for the general researcher. They present a universal equation to determine the cut points for youth, adults and older adults. Their equation is presented for a wide age interval that seems to fail to take maturation into account, evidently with the highest specificity value for the youth cut points being 22.3% (64).

The Romanzini cut points are to the authors knowledge the only cut points that are calibrated for both the vertical axis and vector magnitude from the same data that provides cut-points for SED, LPA, MPA and VPA. They categorized their uniaxial cut

points for SED as <46 counts*15-s (<184 CPM), LPA as <606 counts*15-s (<2427 CPM), MPA as <817 (<3271 CPM) and VPA as >818 counts*15-s (>3272 CPM). Their triaxial cut points for SED was <180 counts*15-s (<720 CPM), LPA as <756 counts*15-s (<3027 CPM), MPA as <1111 (<4447 CPM) and VPA as >1112 counts*15-s (>4448 CPM). The SED and MVPA cut points were similar to the vertical axis cut points of Evenson and colleagues (34). This adds comparability between the uniaxial SED and MVPA cut points of Romanzini and Evenson. Calibration studies often use a portable metabolic system for measuring the metabolic cost of each activity. The Cosmed K4b2 (Cosmed, Rome, Italy) was used in combination with a heart rate transmitter (Polar, Kempele, Finland). The Cosmed has been validated for use for children (61). Their study provides a more detailed calibration protocol with more free-living activity than the aforementioned studies. In addition to walking/running at a treadmill they include sports and activities such as soccer, basketball, volleyball and skipping rope (34). This introduces more movement detectable to the antero-posterior and medio-lateral axis which in turn would be applicable to free living activity registration. When a person walks straight forward the vector magnitude will register more PA than the vertical axis (33), evidently in the calibration protocol (34), but even more during sports (8). The increase in the vertical axis can be up to 6 times higher than the increase in the antero-posterior and medio-lateral axes during walking behavior, which makes the output from the two axes lower than it would be during other activities (33). The activities included in the Romanzini calibration protocol should be considered a strength because the generated counts from the activities are more dependent on the activity than the individuals characteristics. This resulted in almost perfect discrimination (ROC-AUC = >0.93) for SED and all PA intensities (34).

Composite risk score

Despite several ways to define the metabolic syndrome and no consensus on which criteria to use (65), the prevalence in children is increasing (66). While different definitions disagree on the range and thresholds, they agree on which variables to include. The definition mostly includes a measure of fasting or impaired glucose, waist circumference, triglycerides, HDL and blood pressure. These variables are recognized as risk factors for adverse health outcomes later in life, as children with metabolic syndrome have increased risk for having metabolic syndrome in adulthood (67). With

this increased risk follows the risk of all-cause mortality, cardiovascular disease mortality and morbidity, type 2 diabetes and some types of cancer (68). Because a risk factor really have no hard endpoint in children (69), the variables are treated as markers that are used as indicators for cardiometabolic health. For this thesis, the use of and difference between factors and markers should be clarified. A risk factor can be defined as a measurable biological characteristic in an individual that precede a defined outcome of a disease, can predict that disease and is directly associated with that disease trough causality. A marker is a biological indicator that may or may not be causal. The marker can be involved in the process of developing a disease, but it is not necessary for the development. Risk factors have important properties as to identify asymptomatic individuals who are at risk of develop that disease in the future compared to the general population (70). Few children will exhibit any sign of the long term adverse health outcomes before after adolescence, seeing how the metabolic syndrome track from childhood to adulthood (71), metabolic syndrome and the variables included in the definition should be considered risk markers for children (72).

When investigating SED or PAs association to metabolic health, the associations is either individually to one risk marker or in a cluster of risk in composite scores. There is no consensus as to which variables to include in a score, but it should include risk factors that can identify risk and reflects the metabolic syndrome as these variables have predictive capabilities from youth to adulthood (72). Most composite scores consist of one measures of adiposity, blood pressure, triglycerides, total cholesterol (TC), some sort of ratio between lipoprotein subclasses and a measure of insulin/glucose intolerance (69). Some composite scores include an indirect or direct measure of CRF as this has shown to attenuate cardiometabolic risk independent of age and sex (24). There are many variables that represent the same measure which may introduce challenged with multicollinearity. For example, when including body mass index (BMI) or waist circumference as the measure of adiposity in our composite score there was a correlation of r=0.97 for the scores (data not shown). Despite showing almost identical capabilities there are some considerations to make prior to selecting a variable for a composite score. While both measures represent adiposity, each variable measure it differently. BMI uses height and weight to determine a person’s weight status based on pre-established cut-offs. By doing so BMI fails to take lean body or bone mass into account which could result in children with a healthy weight being misclassified as

overweight (73). BMI is not able to measure bodyfat distribution as an indicator of adiposity (74). While suitable for use in populations, individual assessment is not recommended without additional supplementary measures. Waist circumference is a valid measure of abdominal fatness which is related to the accumulation of excess subcutaneous fat storage. As with BMI, it cannot measure bodyfat distribution either. In our sample maturation is a potential confounder that may greatly influence weight, height and body composition. However, the gynoid fat storage on the hips in women have yet to manifest itself at this age (75), therefore making the subcutaneous fat around the waist a valid measurement of bodyfat distribution in children (74). It would be desirable to use a measure of adiposity that could address maturation while taking abdominal adiposity into account. We included waist-to-height ratio (WThR) in our composite score. Given the challenges and the aforementioned measurement properties, the WThR can measure abdominal adiposity while accounting for height differences due to maturation (74). WThR of >0.5 are an efficient threshold of risk in children of all ages (74). The WThR can detect abdominal adiposity and identify children without abdominal adiposity that are categorized as overweight by BMI (76).

Blood pressure is an easy, low cost and non-invasive measure that is routinely included in most epidemiological studies and composite scores. There are different methods of measuring and comparing blood pressure. The average and number of measurement and the final variable used for analysis varies (77). Most studies either use the average of 2-4 measurements for the mean arterial pressure, systolic blood pressure (SBP) or diastolic blood pressure (DBP). High blood pressure tracks from childhood to adulthood, but children rarely have elevated DBP values at a young age. A review and meta-analysis of the tracking of blood pressure found an overall greater tracking correlation for SBP than DBP (78). In composite scores for children, SBP has shown to be a better measure of cardiovascular health than DBP and improves the classification accuracy (79). The mean SBP of three measures are included in our composite score.

CRF is included in our composite score as it is a strong predictor for both individual and clustered risk. CRF will potentially increase over time in healthy children. This is not observed in our children's CRF as this remains relatively stable through the intervention period (7 months) of the Active smarter kids' study. The PA level on the other hand, varies and to account for differences in the samples PA level, CRF is included as this is

a result of long-time activity/inactivity (24). The inclusion of CRF in composite scores is debated. The validity and reliability of the tests used to assess CRF decreases as sample sizes increases moving from direct to indirect tests due to feasibility. Smaller samples allow for direct analysis of respiratory gases involved in pulmonary ventilation, but due to the high cost of equipment, time consumption and required test expertise, indirect tests such as 20-meter shuttle runs are often used for assessing CRF in large population samples. A systematic review of the validity of field tests to estimate CRF concluded that 20-meter shuttle run tests are the most feasible way to evaluate CRF in large samples of children (80). A validation study of The Andersen test concluded that the test provides reliable and valid data on a group level for 10-year-old children, but there was a large individual variability (81). The Andersen test has limitations that should be considered as to why we included CRF in a composite score based on measurement properties. Indirect tests can only be interpreted as an estimation of CRF. The interpretation of units of mL/kg/min is derived from a regression equation that is population specific. The test might penalize those with high body fat and the protocol is susceptible to test familiarization, and are biased by confounding factors such as time, location and inter-rater test variability (81, 82). The testing was done by the same test leaders and there were few children categorized as overweight or obese (14.4 and 3.3% respectively). This should limit the degree of random errors and biases related to the Andersen test results.

Triglycerides, TC and lipoprotein subclasses (typically low-density lipoprotein (LDL) and HDL) are assessed in standard lipid profiles and composite scores. Triglycerides has shown a strong association to cardiovascular disease risk, where those with higher triglycerides have higher risk which is often accompanied with higher levels of LDL and lower HDL (83). There is no association between SED and TC, HDL or LDL and inconsistent evidence that MVPA improves any of the associations (2). The serum levels of TC appear to have a moderate tracking correlation coefficient throughout adolescence (84) and are affected by CRF as higher CRF seems to stimulate a shift towards a less atherogenic lipid profile (85). The combination of triglycerides to represent the free fatty acids in blood plasma and the TC:HDL ratio to represent the total serum concentration and the less atherogenic lipoprotein subclass, has a higher cardiometabolic predicative capability than any of the factors alone (85, 86).

In the international diabetes federation diagnostic criteria for metabolic syndrome a fasting glucose above 5.6 mmol/L is included as an absolute criteria. In a composite score for children, glucose have little predicative capabilities, as they could have developed insulin resistance without the fasting glucose being elevated (69). Insulin resistance is a result of long-term exposure to adiposity and is regarded as a key factor in the development of several adverse health outcomes later in life. Fasting glucose and insulin are used to compute a HOMA score which has shown to be a better measure of impaired glucose regulation than a measure of glucose alone. However, children with insulin resistance are still able to sufficiently regulate their blood glucose. Therefore, the majority of the HOMA score is determined by measure of insulin alone in children. Insulin will be included in our composite score to not miss possible concealed information by variable computation (69, 87).

Most other composite scores include either the same variables or merged variables as a z-score, making our composite score comparable to others (88). Computing a continuous Z-risk score is preferred over dichotomized criteria scores. Example wise, metabolic syndrome in childhood does not predict metabolic syndrome in adulthood when using categorical/dichotomized definition criteria. However, when combining the risk factor criteria into a continuous cluster score, a tracking correlation was shown in early adulthood (72). Dichotomizing criteria can mask important tracking effects and assessing risk as «present» or «absent» does not take clustering into account. Where one participant might move from being above to below a threshold, the relative reduction might be smaller than one who's still are above a threshold after an intervention (72, 89). When constructing a composite score, it assumes that each of the included variables are weighted equally. Some factors might play a more prominent role in predicting or developing of a health-related outcome. While several risk factors might not surpass a defined threshold, one or more variable might be elevated to the point where the risk cluster without being defined as at risk (71).

2. Methods and materials

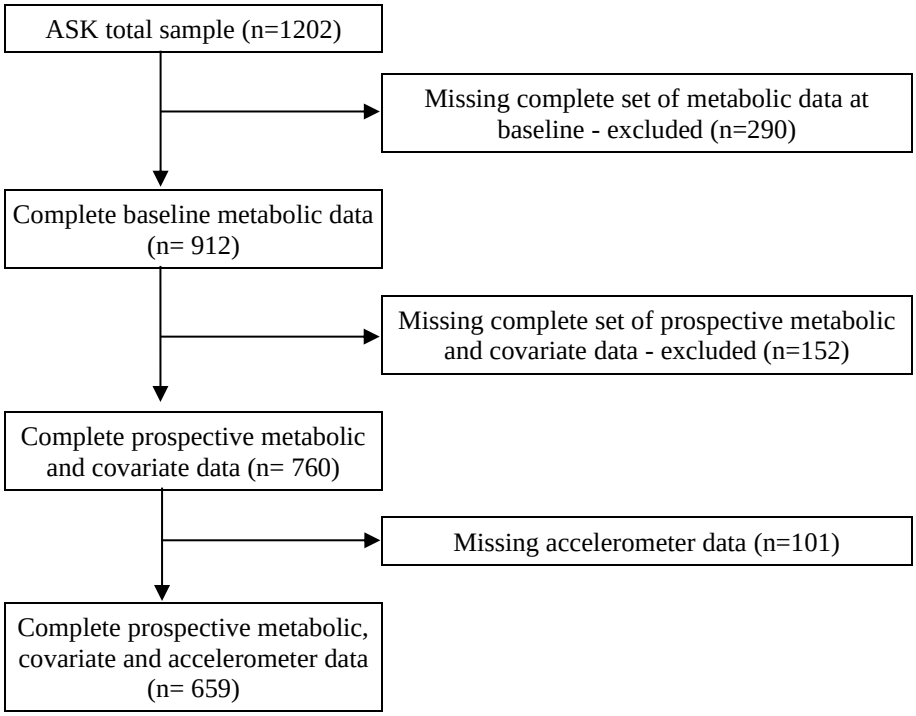
Study design

This study uses data from The Active Smarter Kids Study. The Active Smarter Kids Study is a cluster randomized controlled trial with a parallel group design. They aimed to investigate the effects of a 7-month long PA intervention during school hours on academic performance and cardiometabolic risk markers related to non-communicable diseases. Schools with at least seven children in their 5^{th} grade class, which were without any serious or chronic illnesses, could participate in daily PA, physical education and were able to complete the academic tests were invited to participate. The participants were randomized in a 1:1 ratio by a neutral third party (90). This study uses a longitudinal design with prospective metabolic data collected at baseline and after the intervention period. Accelerometer data was collected before the intervention period at the included schools with the assumption that the baseline PA represents the childrens usual PA levels. The participants with complete data on objectively measured PA, prospective cardiometabolic risk markers and covariate data was included in our study.

Study sample

We used prospective data from The Active Smarter Kids Study which consisted of 1202 fifth-grade children (born in 2004) from 60 schools in Sogn and Fjordane county – Norway. The data was collected over one schoolyear from 2014-15. Three schools declined participation excluding 27 children, leading to a total of 1175 available children, of which 1145 (97.4%) agreed to participate. The final sample represents 86.2% of the total population of 10-year old's in the county, and 95.2% of all eligible for participation. Measures of baseline metabolic variables were available for 1129 (98.6%) children (figure 1) and 1075 (89.4%) provided accelerometer data. A total of 442 children were excluded due to missing pre and/or post metabolic values in our study and additionally 101 for missing accelerometer data, therefore making our study sample consist of 659 children with complete data.

Figure 1: Flowchart of valid and excluded study participants (n=) from baseline to final study sample.



ASK= The Active Smarter Kids Study

Data collection

Anthropometrics and maturation

Body weight was measured to the closest 0.1 kg by an electronic scale (Seca 899, SECA Gmbh, Hamburg, Germany) with participants wearing light clothing. Height was measured to the closest 0.1 cm by a portable stadiometer (Seca 217, SECA Gmbh, Hamburg, Germany), facing forward without shoes. Height and weight were combined into BMI by the formula (kg/m^2). The classification of weight status is based on previously established BMI cut points (91). Waist circumference was measured to the closest 0.1 cm by an ergonomic circumference tape (Seca 201, SECA Gmbh, Hamburg, Germany), approximately 2 cm – in level, over the umbilicus with a relaxed abdomen at the end of a gentle expiration. The mean of two measurements was used unless they varied more than 1 cm, then a third measurement was taken and the two closest measures were used for analysis (90). Waist circumference and height were combined into WThR (Waist circumference/ Height). The WThR accounts for differences in height due to maturation and body fat distribution as it detects central abdominal obesity

in children, both those normal and overweight/obese (76). Pubertal stage was measured by the Tanner method using an image scale (92). Self-assessed pubertal status by children using the Tanner method has previously been validated (93). Measures were conducted in a private room where each participant was shown pictures of pubic hair and genitalia (both sexes), and breast development for girls. The participants read a brief description for each stage and put a checkmark at the picture that best represented their own development. The room had a safe and relaxed atmosphere and the researchers were the same sex as the participant (90). Socio economic status was assessed through a self-reported questionnaire by the guardians/parents. We used the mothers highest achieved education for this study. The categories were; 1) Lower/upper secondary school; 2) University <4 years; 3) University >4 years (90).

Assessment of physical activity

PA was measured by ActiGraph GT3X/GT3X+ accelerometer (ActiGraph, LLC, Pensacola, Florida, USA) worn at the right side of the waist for seven consecutive days. The participants were instructed to remove the accelerometer only during water activities (e.g. swimming and showering) and when sleeping. The ActiGraph GT3X (ActiGraph, LLC, Pensacola, Florida, USA) is a 27 gram, 3.8x3.7x1.8 cm device typically worn on an elastic belt, on the hip. The acceleration sensor in the GT3X is an ADXL335 (Analog devices, Norwood, Massachusetts, USA) which is a surface micromachined, monolithic integrated circuit polysilicon dual axis chip in a microelectron-mechanical-system (MEMS) accelerometer sensor with a dynamic range of ±3g (94). The ActiGraph GT3X+ (ActiGraph, LLC, Pensacola, Florida, USA) weighs 19 gram, measures 4.6x3.3x1.5 cm and uses a Kionix (Ithaca, NY, USA) KXSC7-3672 MEMS accelerometer sensor with ±6g as dynamic range (95). Despite the different MEMS sensors, the principles for recording acceleration are the same. The sensors have two independent polysilicon fixed plates working as electrodes. Parallelly bridging the electrodes is a moveable central plate with polysilicon springs attached to each side of the electrodes forming two back to back capacitors to each fixed plate. These plates form a differential capacitor where the deflection of the central plate responds to acceleration by giving out a specific voltage change to movement. The voltage change is compared to a constant existing electric flow which is an analog signal proportional to the detected acceleration (7). Data was sampled at 30 Hz and

analyzed by using Propero ActiGraph data analyzer version 18 (University of Southern Denmark, Odense, Denmark). We applied a non-wear-time criteria of uninterrupted consecutive 20 min with zero counts (47), ≥480 min/day between 06.00 AM and 00.00 PM as a valid day for ≥4 days/week (44) with the normal filter function as valid monitor data. We analyzed both the vertical axis and vector magnitude in 1, 10 and 60-s epochs by using the Romanzini cut-points of ≤184=SED, ≤2427=LPA, ≤3271=MPA and ≥3272 CPM=VPA for the vertical axis and ≤720=SED, ≤3027=LPA, ≤4447=MPA and ≥4448 CPM=VPA for the vector magnitude (34). The epochs were chosen to accommodate shorter epochs for children and enable cross time comparisons. The earliest ActiGraph uniaxial accelerometers collected data at a 60-s epoch due to storage capabilities, in contrast to the more modern ones that are not restricted by storage space (e.g. <15-s epochs). Additionally, we contribute to the further expansion on the ongoing research in axial differences by using similar processing criteria as previous research (10). The cut points are originally calibrated in a 15-s epoch length, so the cut points were reintegrated into 1, 10 and 60-s epochs by multiplying by four and then dividing by 6 and 60.

Blood pressure

Blood pressure was measured by an Omron HBP – 1300 automated monitor (Omron healthcare, inc, Vernon hills, IL, US) after a 10-min rest, in a sitting position without distractions. The monitor has been validated for blood pressure measurements (96). Measurements were taken in a quiet room on the upper right arm with a size appropriate cuff. They measured blood pressure four times with 1 minute between each measurement, with the mean of the three last measures being used, unless they varied more than 5 mmHg, then a fifth measure was taken and the mean of the four last measures being used for analysis (90).

Blood samples

All samples were collected by a nurse or phlebotomist by intravenous blood from the antecubital vein, after an overnight fast, between 08.00 -10.00 AM in the morning. The samples were obtained following a 5 step process: 1. Blood plasma was sampled in 5 mL tubes with gel (Vakuette® serum gel with activator, G456073); 2. The tubes were turned upside-down five times before being placed vertically for coagulation; 3. 30-min

later the tubes was centrifuged at 2000 g for 10-min. This process was repeated until there was no visual residues present; 4. The samples were stored at 4°C before pipetting 0.5 ml into cryo tubes; 5. The samples were stored at -80°C before biochemistry analysis (90). The samples were analyzed for insulin, glucose, triglycerides, TC, HDL and LDL by standard laboratory methods. Both pre and post intervention samples were analyzed at the same time at the accredited endocrine laboratory of the VU medical center (VUmc, Amsterdam, the Netherlands). Analysis of low molecular weight metabolites and lipoprotein subclasses was done by proton magnetic resonance (1H-NMR) spectroscopy. The proton NMR profiles of the fatty and amino acids were obtained by using a standard experimental setup (97). Lipoproteins were obtained by a minor modified protocol as used by (98). Each average lipoprotein subclass concentration of each class was derived from NMR profiles. Fatty acids were analyzed by following protocol: a 200 µL blood samples were weighted into a 10mL glass tube and water evaporated under nitrogen, followed by adding 150 µL internal standard (triheptadecanoin, 0.4855mg/mL). after evaporation the sample was dervatized to fatty acids methyl esters (FAME) by direct esterification in methanolic HCI at 90°C for 2h under nitrogen atmosphere (99). FAME was then extracted and analyzed by gas chromatography as previously described (100). The samples were run in a randomized sequence and the FAME reference mixture GLC-461 (Nu-Chek prep, Elysian, MN, US) was analyzed as every 10^{th} sample. Chromatographic areas were corrected by empirical response factors calculated from the GLC-461 mixture. The amount of fatty acids was quantified by means of the internal standard. The total amount in each sample was converted to amounts in µg per g sample by dividing by the sample weight.

Cardiorespiratory fitness

CRF was assessed by The Andersen test which is an intermittent running test. Participants were allowed a 10-min warm-up and received information about the protocol prior to the test. Two parallel lines were drawn 20-m apart in a gymhall or similar accommodations, with a wooden or rubber floor. Participants started at one line, ran to the other where they had to touch the floor behind the line with one hand before turning and running back (101). The test was divided into continuous 15-s periods of running and resting (standing still) separated by the test leaders whistle blow, for a total of 10-min (e.g. 5 minutes of breaks and 5 minutes of running). Participants ran as fast as

possible and tried to cover the longest distance they could. At the last whistle blow participants stopped as fast as possible (about 2 steps) and their distance was measured and summed as the test result. Each group consisted of 10-20 children where one member of the research staff was responsible for registration of 1-2 children. Verbal encouragements were given throughout the test (101). The relationship between the Andersen test and physical fitness has shown an overall ICC=0.84. The bivariate relationship between the Andersen test and $Vo_{2\text{-peak}}$ were r=0.73. The Andersen test provides reliable and valid data on a group level for 10-year-olds (81).

Statistics

Descriptive statistics of anthropometrics, demographics, cardiometabolic risk variables and the amount of time spent sedentary and in different PA intensities are presented as mean with standard deviation, unless stated otherwise. Continuous data variables were tested for normal distribution through Kolmogorov/Smirnov test of normality and visual inspection of histogram distribution. The difference between included and excluded participants were tested through independent sample t-tests. Categorical variable differences were tested through persons chi square tests and uni- and triaxial SED and PA differences through paired sample t-tests. The correlation between uni- and triaxial mean CPM in different epoch lengths are illustrated through correlation plots. We computed a composite cardiometabolic risk score by summing the z-scores ($\frac{\text{Sum}-\text{mean}}{\text{standard deviation}}$) of WThR, SBP, triglycerides, TC:HDL ratio, CRF (Andersen test in meters) and insulin. The magnitude of association between SED and different PA intensities and the cardiometabolic risk score was assessed through multiple linear regression models. The model was adjusted for age, sex, maturity, socio economic status, wear-time and the baseline cardiometabolic risk score. Additionally, SED was controlled for MVPA and MPA, VPA and MVPA for SED. we tested for significant difference in the regression slopes between the uni- and triaxial estimates of the same epoch length by using their beta-coefficients and standard error. Based on the assumption that the standard error variance were heterogeneous and that each independent variable in the regression model had >20 observations we tested for difference by using the formula $z = \frac{\beta 1 - \beta 2}{\sqrt{SE1^2 - SE2^2}}$. A z-value of ± 1.96 represents a statistical difference (102). The dose-response patterns of MVPA and the cardiometabolic risk score were assessed through mixed regression models. The models

were adjusted for the same variables as the previous models and SED. MVPA were divided into quartiles and used as the fixed effect, while school were used as the random effect. All statistical analyses were done in Statistical package of social science version 24 (SPSS, INC, Chicago, Illinois, USA). The alpha level was set to $p=\leq 0.05$.

Ethics

The Active Smarter Kids Study was registered at clinicaltrials.gov (NCT02132494) and was approved by the south-east regional committee for medical research ethics in Norway. Data collection and storing was approved by Norwegian center for research data. The research was done in accordance with the world medical associations declaration of Helsinki. All the childrens parent/guardian provided a written informed consent prior to all testing and the children were given a verbal invitation and information about the study. Our project used unidentified data for analysis, with no possibility for the researchers to identify the participants. The dataset used for this thesis will be deleted after final evaluation and publication.

References

1. Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput JP, Janssen J, Katzmarzyk PT. et al. Systematic review of the relationship between objectively measured physical acitivity and health indicators in school-aged children and youth. appl physiol nutr metab. 2016;41:197-239.
2. 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 faktors in youth: a systematic review and meta-analysis. Obes Rev. 2019;20:55-74.
3. Wareham NJ, Rennie KL. Theassessment of physical activity in individuals and populations: why try to be more precise about how physical activity is assessed? Int J obes. 1998;22(2):30-8.
4. Wijndaele K, Westgate K, Stephens SK, Blair SN, Bull FC, Chastin SFM. et al. Utilization and harmonization of adult accelerometry data: Review and expert consensus. Med Sci Sports Exerc. 2015;47(10):2129-39.
5. Fridolfsson J, Börjesson M, Arvidsson D. A biomechanical re-examination of physical activity measurements with accelerometers Sensors. 2018;18(3399).
6. Migueles JH, Cadenas-Sanchez C, Ekelund U, Nystrøm CD, Mora-Gonzalez J, Løf M. et al. Accelerometer data collection and processing criteria to assess physical activity and other outcomes: a systematic review and practical consideration Sports Med 2017;47:1821-45.
7. John D, Freedson P. Actigraph and actical physical activity monitors: A peek under the hood. Med Sci Sports Exerc. 2012;44(1):86-9.
8. Smith MS, Horsch A, Standl M, Heinrich J, Schulz H. Uni- and triaxial accelerometric signals agree during daily rutine, but show differences between sports Sci Rep. 2018;8(15055).
9. Chomistek AK, Yuan C, Matthews CE, Troiano RP, Bowles HR, Rood J. et al. Physical activity assessment with the actigraph GT3X and doubly labeled water. Med Sci Sports Exerc. 2017;49(9):1935-44.
10. Aadland E, Kvalheim OM, Anderssen SA, Resaland GK, Andersen LB. The triaxial physical activity signature associated with meatbolic health in children. Med Sci Sports Exerc. 2019;51(10).
11. Hanggi JM, Phillips LRS, Rowlands AV. Validation of the GT3X actigraph in children and comparison with the GT1M actigraph. J Sci Med Sports. 2012;16:40-4.
12. Smith M, Standl M, Heinrich J, Schulz H. Accelerometric estimates of physical activity vary unstably with data handling. PLoS ONE. 2017;12(11):e0187706.
13. Sagelv EH, Ekelund U, Pedersen S, Brage S, Hansen BH, Johansson J. et al. Physical activity levels in adults and elderly from triaxial and uniaxial accelerometry. The Tromsø study. PLoS ONE. 2019;14(12):e0225670.
14. Kelly LA, McMillan DGE, Anderson A, Fippinger M, Fillerup G, Rider J. Validity of actigraphs uniaxial and triaxial accelerometers for assessment of physical activity in adults in laboratory conditions. BMC Med Phys 2013;13(5).
15. Biddle SJH, Bengoechea EG, Wiesner G. Sedentary behaviour and adiposity in youth: a systematic review of reviews and analysis of causality. Int J Behav Nutr Phy. 2017;14(43).
16. Sedentary behavior research network. Letter to the editor: Standardized use of the terms "sedentary" and "sedentary behaviors". appl physiol nutr metab. 2012;37:540-42.

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(7):1360-8.
18. Tarp J, Brønd JC, Andersen LB, Møller NC, Froberg K, Grøntved A. Physical activity, sedentary behaviour, and long-term cardiovascular risk in young people: A review and discussion of methodology in prospective studies. J Sports Health Sci 2016;5:145-50.
19. Carson V, Ridgers ND, Howard BJ, Winkler EAH, Healy GN, Owen N. et al. Light-intensity physical activity and cardiometabolic biomarkers in US adolescents PLoS ONE. 2013;8(8):e71417.
20. Fedewa MV, Gist NH, Evans EM, Rishman RK. Exercise and insulin resistance in youth: A meta-analysis. Pediatrics. 2014;133(1).
21. Marson EC, Delevatti RS, Garcia Prado AK, Netto N, Kruel LFM. Effects of aerobic, resistance, and combined exercise training on insulin resistance markers in overweight or obese children and adolescents: A systematic review and meta-analysis. Prev Med. 2016;93:211-18.
22. Chinapaw M, Klakk H, Møller NC, Andersen LB, Altenburg T, Wedderkopp N. Total volume versus bouts: prospective relationship of physicalactivity and sedentary time with cardiometabolic risk in children. Int J obes. 2018;42:1733-42.
23. Vaisto J, Haapala EA, Viitasalo A, Schnurr TM, Kilpelainen TO, Karjalainen P. et al. Longitudinal associations of physical activity and sedentary time with cardiometabolic risk factors in children. Scand J Med Sci Sports. 2019;29(1):113-23.
24. Anderssen SA, Cooper AR, Riddoch C, Sardinha LB, Harro M, Brage S, Andersen LB. Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. Eur J Prev Cardiol. 2007;14:526-31.
25. Steele RM, Brage S, Corder K, Wareham NJ, Ekelund U. Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. j appl physiol. 2008;105:342-51.
26. Andersen LB, Sardinha LB, Froberg K, Riddoch CJ, Page AS, Anderssen SA. Fitness, fatness and clustering of cardiovascular risk factors in children from Denmark, Estonia and Portugal: The European youth heart study. Int J Pediatr Obes. 2008;3:58-66.
27. Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S, European youth heart study group Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. Diabetologica. 2007;50:1832-40.
28. Cliff DP, Hesketh KD, Vella SA, Hinkley T, Tsiros MD, Ridgers ND. et al. Objectively measured sedentary behaviour and health and development in children and adolescents: systematic review and meta-analysis. Obes Rev. 2016;17:330 - 44
29. Carson V, Hunter S, Kuzik N, Gray CE, Poitras VJ, Chaput JP. et al. Systematic review of sedentary behaviour and health indicators in school-aged children and youth: an update. appl physiol nutr metab. 2016;41:240-65.
30. Fröberg A, Raustorp A. Objectively measured sedentary behaviour and cardio-metabolic risk in youth: a review of evidence. Eur J Pediatr. 2014;173:845-60.
31. Brooke HL, Atkin AJ, Corder K, Brage S, van Sluijs EMF. Frequency and duration of physical activity bouts in school-aged children: a comparison within and between days. Prev Med Rep. 2016;4:585-90.
32. van Hees VT, Gorzelniak L, Leon ECD, Eder M, Pias M, Taherian S. et al. Separating movement and gravity components in an acceleration signal and

implications for the assessment of human daily physical activity. PLoS ONE. 2013;8(4):e61691.
33. John D, Miller R, Kozey-Keadle S, Caldwell G, Freedson P.Biomechanical examination of the plateau phenomenon in actigraph vertical activity counts Physiol Meas. 2012;33(2):219-30.
34. Romanzini M, Petroski EL, Ohara D, Dourado AC, Reichert FF. Calibration of actigraph GT3X, actical and RT3 accelerometers in adolescents Eur J Sports Sci. 2014;14(1):91-9.
35. Fukuchi CA, Fukuchi RK, Duarte M. Effects of walking speed on gait biomechanics in healthy participants: a systematic review and meta-analysis. Syst Rev. 2019;8(153).
36. Brønd JC, Aadland E, Andersen LB, Resaland GK, Anderssen SA, Arvidsson D. The actigraph counts processing and the assessment of vigrous activity. clin physio funct imaging. 2019.
37. Fridollfsson J, Börjesson M, Buck C, Ekblom Ø, Ekblom-Bak E, Hunsberger M. et al. Effects of frequency filtering on intensity and noise in accelerometer-based physical activity measurements. Sensors. 2019;19(2186).
38. Fairclough SJ, Noonan R, Rowlands AV, van Hees V, Knowles Z, Boddy LM. Wear compliance and activity in children wearing wrist- and hip- mounted accelerometers. Med Sci Sports Exerc. 2016;48(2):245-53.
39. Tudor-Locke C, Barreira TV, Schuna jr JM, Mire EF, Chaput JP, Fogelholm M. et al. Improving wear time compliance with a 24-hour waist-worn accelerometer protocol in the international study of childhood obesity, lifestyle and the enviroment (ISCOLE). Int J Behav Nutr Phy. 2015;12(11).
40. Hildebrand M, van Hees VT, Hansen BH, Ekelund U. Age group comparability of raw accelerometer outputs from wrist- and hip-worn monitors. Med Sci Sports Exerc. 2014;46(9):1816-24.
41. Hildebrand M, Hansen BH, van Hees VT, Ekelund, U. Evaluation of raw acceleration sedentary threshold in children and adults. Scand J Med Sci Sports. 2017;27:1814-23.
42. Troiano RP, McClain JJ, Brychta RJ, Chen KY. Evolution of accelerometer methods for physical activity research. Br J Sports Med. 2014;48(13):1019-23.
43. White T, Westgate K, Hollidge S, Venables M, Olivier P, Wareham N, Brage S. Estimating energy expenditure from wrist and thigh accelerometry on free-living adults: a doubly labelled water study. Int J obes. 2019;43:2333-42.
44. Cain KL, Sallis JF, Conway TL, van Dyck D, Calhoon L. Using accelerometers in youth physical activity studies: A review of methods. J Phys Act Health. 2013;10(3):437-50.
45. Aadland E, Andersen LB, Anderssen SA, Resaland GK. A comparison of 10 accelerometer non-wear time criteria and logbooks in children. BMC Public Health. 2018;18(323).
46. Atkin AJ, Ekelund U, Møller NC, Froberg K, Sardinha LB, Andersen LB, Brage S. Sedentary time in children: Influence of accelerometer processing on health relations. Med Sci Sports Exerc. 2013;45(6).
47. Banda JA, Haydel KF, Davila T, Desai M, Bryson S, Haskell WL. et al. Effects of varying epoch lengths, wear time algorithms, and activity cut-point on estimates of children sedentary behavior and physical activity from accelerometer data. PLoS ONE. 2016;11(3):e0150534.

48. Toftager M, Kristensen PL, Oliver M, Duncan S, Christiansen LB, Boyle E. et al. Accelerometer data reduction in adolescents: effects on sample retention and bias. Int J Behav Nutr Phy. 2013;10(140).
49. VanHelst J, Vidal F, Drumez E, Beghin L, Baudelet JB, Coopman S, Gottrand F. Comparison and validation of accelerometer wear time and non-wear time algorithms for assessing physical activity levels in children and adolescents. BMC Med Res Methodol 2019;19(72).
50. Brønd JC, Arvidsson D. Sampling frequency affects the processing of actigraph raw acceleration data to activity counts. j appl physiol. 2015;120:362-69.
51. Hjorth MF, Chaput JP, Damsgaard CT, Dalskov SM, Michaelsen KF, Tetens I, Sjödin A. Measure of sleep and physical activity by a single accelerometer: can a waist worn ActiGraph adequately measure sleep in children? . Sleep biol rhythms. 2012;10:328-35.
52. Aadland E, Andersen LB, Anderssen SA, Resaland GK, Kvalheim OM. Associations of volumes and patterns of physical activity with metabolic health in children: A multivariate pattern analysis approach. Prev Med. 2018;115:12-8.
53. Kim Y, Beets MW, Pate RR, Blair SN. The effect of reintegrating actigraph accelerometer counts in preschool children: Comparison using different epoch lengths. J Sci Med Sports. 2012;16(2013):129-34.
54. Logan GRM, Duncan S, Harris NK, Hinckson EA, Schofield G. Adolescent physical activity levels: discrepancies with accelerometer data analysis. J Sports Sci. 2016;34(21):2047-53.
55. Sanders T, Cliff DP, Lonsdale C. Measuring adolescent boys physical activity: bout length and the influence of accelerometer epoch length. PLoS ONE. 2014;9(3):e92040.
56. Aibar A, Bois JE, Zaragoza J, Generelo E, Julian JA, Paillard T. Do epoch lenghts affect adollescents compiance with physical activity guidelines. J Sports Med Phys Fitness. 2014;54(3):324-34.
57. Aadland E, Andersen LB, Anderssen SA, Resaland GK, Kvalheim OM. Accelerometer epoch setting is decisive for associations between physical activity and metabolic health in children. J Sports Sci. 2019.
58. Aibar A, Chanal J. Physical education: The effect of epoch lenghts on childrens physical activity in a structured context. PLoS ONE. 2015;10(4):e0121238.
59. 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;44:143-47.
60. Migueles JH, Cadenas-Sanchez C, Tudor-Locke C, Løf M, Esteban-Cornejo I, Molina-Garcia P. et al. Comparability of published cut-points for the assessment of physical activity: implications for data harmonization Scand J Med Sci Sports. 2018:1-9.
61. Harrell JS, McMurray RG, Baggett CD, Pennell ML, Pearce PF, Bandiwala SI. Energy costs of physical activities in children and adolescents. Med Sci Sports Exerc. 2005;37(2):329-36.
62. 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(14):1557-65.
63. Jimmy G, Seiler R, Mader U. Development and validation of GT3X accelerometer cut-off points in 5-to9-year-old children based on indirect calorimetry measurements Schweiz z Med Traumatol. 2013;61(4):37-43.

64. Santos-Lozano A, Santin-Medeiros F, Cardon G, Torres-Luque G, Bailon R, Bergmeir C. et al. Actigraph GT3X: Validation and determination of physical activity intensity cut points. Int J Sports Med 2013;34:975-82.
65. Zimmet P, George K, Alberti MM, Kaufman F, Tajima N, Silink M. et al. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatr diabetes. 2007;8:299-306.
66. Moore JX, Chaudhary N, Akinyemiju T. Metabolic syndrome prevalence by race/ethnicity and sex in the United states, national health and nutrition examination survey 2988-2012. Prev chronic dis. 2017;14(e24).
67. Viitasalo A, Lakka TA, Laaksonen DE, Savonen K, Lakka HM, Hassinen M. et al. Validation of metabolic syndrome score by confirmatory factor analysis in children and adults and prediction of cardiometabolic outcomes in adults. Diabetologica. 2014;57:940-49.
68. Ford ES. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome. Diabetes care. 2005;28(7):1769-78.
69. Andersen LB, Lauersen JB, Brønd JC, Anderssen SA, Sardinha LB, Steene-Johannessen J. et al. A new approach to define and diagnose cardiometabolic disorder in children. J Diabetes Res. 2015.
70. Balagopal P, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL. et al. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth. Circulation. 2011;123:2749-69.
71. Eisenmann JC, Laurson KR, DuBose KD, Smith BK, Donnelly JE. Construct validity of a continuous metabolic syndrome score in children. Diabetol Metab Syndr. 2010;2(8).
72. Beckstead JW, Beckie TM. How much information can metabolic syndrome provide? An application of information theory. Med decis making. 2011;31:79-92.
73. Mei Z, Grummer-Strawn lM, Pietrobelli A, Goulding A, Goran MI, Dietz WH. Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. Am J Clin Nutr. 2002;75:978-85.
74. Horan M, Gibney E, Mooloy E, McAuliffe F. Methodologies to assess paediatric adiposity. Ir J Med Sci. 2015;184:53-68.
75. Pozza Santos L, Santos IS, Matijasevich A, Barros AJD. Changes in overall and regional body fatness from childhood to early adolescence. Sci Rep. 2018;9(1888).
76. Mokha JS, Srinivasan SR, DasMahapatra P, Fernandez C, Chen W, Xu J, Berenson GS. Utility of waist-to-height ratio in assessing the status of central obesity and related cardiometabolic risk profile among normal weight and overweight/obese children: The Bogalusa heart study. BMC pediatr. 2010;10(73).
77. Feber J, Ahmed M. Hypertension in children: new trends and challenges. Clin Sci. 2010;119:151-61.
78. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: A systematic review and meta-regression analysis. Circulation. 2008;117(25):3171-80.
79. Lerum Ø, Aadland E, Andersen LB, Anderssen SA, Resaland GK. Validity of noninvasive composite scores to assess cardiovascular risk in 10-year-old children. Scand J Med Sci Sports. 2016;27:865-72.
80. Batista MB, Romanzini CLP, Castro-Pinero J, Vaz Ronque ER. Validity of field tests to estimate cardiorespiratory fitness in children and adolescents: A systematic review. Rev Paul Pediatr. 2017;35(2):222-33.

81. 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(10):e110492.
82. Armstrong N, Welsman J. Twenty-metre shuttle run: (mis) representation, (mis) interpretation and (mis) use. Br J Sports Med. 2019;53(19):1199-200.
83. Kannel WB, Vasan RS. Triglycerides as vascular risk factors: New epidemiologic insights for current opinion in cardiology Curr Opin Cardiol. 2009;24(4):345-50.
84. Nicklas TA, von Duvillard SP, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: The Bogalusa heart study. Int J Sports Med. 2002;23:39-43.
85. 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. 2017;28:220-27.
86. Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J. et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55000 vascular deaths. Lancet. 2007;370:1829-39.
87. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J. et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA. 2014;311(17):1778-86.
88. Kamel M, Smith BT, Wahl G, Carsley S, Birken CS, Anderson LN. Continuous cardiometabolic risk score definitions in early childhood: a scooping review. Obes Rev. 2018;19:1688-99.
89. Kelly AS, Steinberger J, Jacobs jr DR, Hong CP, Moran A, Sinaiko AR. Predicting cardiovascular risk in young adulthood from metabolic syndrome, its component risk factors, and a cluster score in childhood. Int J Pediatr Obes. 2011;6(0):283-89.
90. Resaland GK, Moe VF, Aadland E, Steene-Johannessen J, Glosvik Ø, Andersen JR. et al. Active smarter kids (ASK): rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on childrens academic performances and risk factors for non-communicable diseases. BMC Public Health. 2015;15(709).
91. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. BMJ. 2000;320:1-6.
92. Carel JC, Leger J. Precocious puberty. N Engl J Med. 2008;258:2366-77.
93. Chavarro JE, Watkins DJ, Afeiche M, Zhang Z, Sanchez BN, Cantonwine D, Peterson KE. Validity of self-assessed sexual maturation against physician assessments and hormone levels. J Pediatr. 2017;186:172-78.
94. Analog-devices-Norwood-MA-U.S.A. Small, low power, 3-axis ±3g accelerometer. 2009. p. 16p.
95. Kionix-Ithaca-NY-U.S.A. ±6g Tri-axis analog accelerometer spesifications. 2015. p. 13p.
96. El Assaad MA, Topouchian JA, Darne BM, Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood pressure monitoring. Blood press monit. 2002;7(4):237-41.
97. Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R. et al. High-throughput serum NMR metabonomics for cost- effective holistic studies on systemic metabolism. Analyst. 2009;134(9):1781-85.

98. Mihaleva VV, van Schalkwijk DB, de Graaf AA, van Duynhoven J, van Dorsten FA, Vervoort J. et al. A systematic approach to obtain validated partial least square models for predicting lipoprotein subclasses from serum NMR spectra. Anal chem. 2014;86(1).
99. Meier S, Mjøs SA, Joensen H, Grahl-Nielsen O. Validation of a one-step extraction/methylation method for determination of fatty acids and cholesterol in marine tissues. J Chromatogr. 2006;1104:291-98.
100. Gudbrandsen OA, Kodama, Y., Mjøs, S. A., Zhao, C. M., Johannessen, H., Brattbakk, H. R. et al. Effects of duodenal switch alone or in combination with sleeve gastrectomy on body weight and lipid metabolism in rats. Nutr Diabetes. 2014;4:e124.
101. Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. J Sport Med Phys Fit. 2008;48(4):434-7.
102. Kleinbaum DG, Kupper LL, Nizam A, Rosenberg ES. Applied regression analysis and other multivariable methods. 5th ed. Boston: Cengage Learning; 2014.

Article

Title

Physical activity measured by uni- or triaxial accelerometry - Does it matter for the association to cardiometabolic risk markers in children?

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Word count: 6140 (including abstract)

List of abbreviations: PA= Physical activity, LPA= Light physical activity, MPA= Moderate physical activity, VPA= Vigorous physical activity, MVPA= Moderate-to-vigorous physical activity, CPM= Counts per minute, MET= metabolic equivalent of task, HDL= high density lipoprotein, LDL= Low density lipoprotein, WC= Waist circumference.

Declarations

Ethics approval and consent to participate

The Active Smarter Kids study was approved by the south-east regional committee for medical research ethics in Norway and registered at clinicaltrials.gov (NCT02132494). Data collection and storing was approved by Norwegian center for research data. The research was done in accordance with the world medical associations declaration of Helsinki. All the childrens parent/guardian provided a written informed consent prior to all testing and the children were given a verbal invitation and information about the study

Consent for publication

Not applicable

Availability of data and materials

The dataset used in this study are available from the Active Smarter Kids publication group upon reasonable request

Competing interests

The authors declare they have no competing interests

Funding

The study was supported by grants from the Research Council of Norway (grant number 221047/F40 and 249932/F20), the Gjensidige Foundation (grant number 1042294), and the Western Norway University of Applied Sciences (formerly credited as the Sogn og Fjordane University College)

Authors' contributions

UE and JT conceived the study. TEN performed the analyses and wrote the first draft. UE and JT read and critically reviewed the manuscript. All authors approved the final manuscript.

Acknowledgements

The authors gratefully acknowledge the work of numerous master students and PhD candidates who have participated in the data collection for the Active Smarter Kids Study. We thank the children, parents/guardians, and teachers in the schools involved in the project.

Abstract

Background: Differences in physical activity measured by uni- and triaxial accelerometry and their magnitude of association to metabolic health and the influence of epoch length in children is unknown**.**

Methods: We used data from a 7-month school-based randomized controlled trial conducted over the school year 2014-2015 in Sogn & Fjordane county in Norway. Data were available for a total of 1129 children of which 659 had complete physical activity, prospective cardiometabolic and covariate data. We applied the Romanzini cut points to the accelerometer data. These cut points provide thresholds for both uni- and triaxial estimates that are calibrated from the same data. Time spent sedentary, light, moderate, vigorous, and moderate-to-vigorous was then analyzed in 1, 10 and 60-s epoch length. We analyzed the prospective association between a cardiometabolic risk score and sedentary time, light, moderate, vigorous, moderate-to-vigorous physical activity by linear regression models. We used mixed regression models to describe the dose-response patterns of moderate-to-vigorous physical activity to cardiometabolic risk. We computed a composite cardiometabolic risk score by summing the z-scores of waist-to-height ratio, systolic blood pressure, triglycerides, total cholesterol: high density lipoprotein ratio, insulin and cardiorespiratory fitness.

Results: All physical activity estimates where significantly different between the uni- and triaxial data reduction. At most, this led to a relative difference of 42.5% higher physical activity guideline compliance. The uni- and triaxial estimates of sedentary time and physical activity associations to metabolic health does not follow any general pattern. The dose- response patterns of moderate-to-vigorous physical activity were similar, but the magnitude of association were greater between the least and most active quartile when measured uniaxial.

Conclusion: Triaxial physical activity estimates show lower prospective associations to cardiometabolic health, especially for moderate, vigorous and moderate-to-vigorous physical activity. The uni- and triaxial difference in the prospective associations is

attenuated in longer epoch lengths. The dose-response patterns of MVPA are similar between the number of axes used and epoch length, but the magnitude of association between the least and most active quartile are greater when measured uniaxial.

Trial registration: The Active Smarter Kids Study was registered at clinicaltrials.gov (NCT02132494).

Keywords: Uniaxial, Triaxial, Accelerometry, Cardiometabolic health, Risk markers, Children.

Introduction

Physical activity (PA), especially when performed with high intensity, is favorably associated with metabolic health markers in children (1,2). PA has historically been assessed by questionnaires, more recently being replaced by accelerometers as a feasible method in large-scale epidemiological studies, where ActiGraph are the most frequently used brand (3). The early accelerometers where uniaxial (vertical axis), but newer models, such as the GT3X+, can record PA on a triaxial basis (medio-lateral, vertical, and antero-posterior axis). In a triaxial output the axes are combined into a vector magnitude proposed as a metric capturing the full range and complexity of human movement. In a vector magnitude the vertical axis explains most of the activity with up to 95% of the variance during daily routine movements (4).

A study recently suggested different associations between PA-metrics and metabolic health when comparing uni- with triaxial accelerometry in children. They found a lower model fit for uniaxial (R^2=17.0%) compared to triaxial (R^2=18.9%) accelerometry (5). Triaxial accelerometry results in significantly less time spent sedentary and more time

spent in both LPA and MVPA compared with estimates derived from uniaxial data (6). The higher amount of MVPA recorded with triaxial accelerometry results in higher PA guideline compliance. Triaxial accelerometry have higher correlation to doubly labelled water determined PA energy expenditure than uniaxial (7) which could imply a future use for triaxial accelerometry in PA research. Triaxial accelerometry is expected to record higher amounts of PA and lower amounts of sedentary time. However, it remains uncertain if this is due to differences in post-processing data decisions or the triaxial signature itself, and if it leads to the magnitude of association between uni- and triaxial measured PA and cardiometabolic risk to differ. The epoch length influences both uni- and triaxial accelerometry classification of different PA estimates. When the epoch length increases the amount of sedentary time, VPA and MVPA decreases, while LPA and MPA increases (8). The magnitude of associations could be influenced by epoch length, but it is unclear how the epoch length affects the uni- or triaxial estimates.

The difference in sedentary time and PA estimates and how this affects the associations with health outcomes in children is unexplored and little is known about the practical implications of uni- and triaxial differences in different epoch length. It is important to determine how this affects associations to metabolic health and if dose-response patterns are similar between the number of axes used and different epoch length. Our primary aim is to investigate; 1) Is there a difference in the magnitude of the prospective associations between uni- and triaxial accelerometry with cardiometabolic health markers in children; secondary aims are 2) Does epoch length affect the magnitude of the prospective associations between uni- and triaxial accelerometry with cardiometabolic health markers; and 3) Does uni- and triaxial accelerometry affect the MVPA dose-response patterns to cardiometabolic health markers in different epoch lengths.

Methods

Participants

We included 1129 5th grade children from the active smarter kids' study. The active smarter kids' study is a 7-month clustered-randomized controlled trial conducted in Sogn & Fjordane county in Norway during the school year 2014-2015 (9). PA was measured at baseline before intervention start by accelerometry and metabolic variables at baseline and post intervention. The children with complete PA data, prospective cardiometabolic and covariate data are included in our analysis.

This study follows the ethical guidelines of the world medical associations declaration of Helsinki. The active smarter kids study protocol was approved by the regional committee for medical research ethics. Written consent was obtained from the children's parents/guardians and from the school authorities before the project start. The active smarter kids' study is registered at Clinical-trials.gov (ID: NCT02132494).

Procedures

A detailed description of the study has been published earlier (9), but we provide a brief description of the methods and procedures for this study. PA was measured by ActiGraph GT3X/GT3X+ accelerometers (ActiGraph, LLC, Pensacola, Florida, USA). The children were instructed to wear the accelerometers on the right side of their hip during awake hours for seven consecutive days, only to remove it during water activities. The accelerometers were set to sample data at 30 Hz. We applied a non-wear-time criteria of 20 min of consecutive zero counts. A valid day was considered ≥480 min/day of recorded acceleration between 06.00 AM and 00.00 PM, where those who

achieved ≥4 days/week was included. We used the normal filter function (standard ActiGraph filter). We analyzed both the vertical axis and vector magnitude in 1, 10 and 60-s epoch length and applied the cut-points proposed by Romanzini and colleagues (10) to define intensity thresholds. The cut points were selected because they provide cut points for the vertical axis and vector magnitude that are both calibrated from the same data applicable to children, thus enabling a direct comparison. Cut-offs for sedentary time, LPA, MPA and VPA were ≤184, ≤2427, ≤3271 and ≥3272 counts per minute (CPM) for the vertical axis and ≤720, ≤3027, ≤4447 and ≥4448 CPM for the vector magnitude. Both set of cut points showed almost perfect sensitivity (>96.9% for the vertical axis and >97.8% for the vector magnitude) and excellent specificity (>84.9% for the vertical axis and >83.7% for the vector magnitude). The cut points are originally calibrated in a 15-s epoch length, so we reintegrated them into 1, 10 and 60-s epoch length by multiplying by four and then dividing by 6 and 60 (exact cut-offs shown in supplementary file). We chose the 1 and 10-s epoch length based on the recommendation of shorter lengths to capture children's sporadic and intermittent PA pattern (11). There are advantages to both length, where there is a tradeoff in capturing different PA intensities. For example, where 1-s epoch would capture every second of MVPA during an activity, it could also capture seconds of sedentary time during passive phases of the same activity. In this case 10-s epoch would aggregate the passive phases with the active, resulting in the entire interval being classified as activity. The 60-s epoch was selected to enable historical comparison to earlier accelerometers that were limited by storage capabilities. We analyzed the accelerometry data using Propero ActiGraph data analyzer version 18 (University of Southern Denmark, Odense, Denmark) (11).

Body weight was measured to the closest 0.1 kg by an electronic scale (Seca 899, SECA Gmbh, Hamburg, Germany) with participants wearing light clothing. Height was measured to the closest 0.1 cm by a portable stadiometer (Seca 217, SECA Gmbh, Hamburg, Germany), facing forward without shoes. Body mass index (BMI, kg/m^2) was calculated from height and weight and weight status classified according to the cut off points proposed by Cole et al (13). Waist circumference (WC) was measured to the closest 0.1 cm by an ergonomic circumference tape (Seca 201, SECA Gmbh, Hamburg, Germany), approximately 2 cm – in level, over the umbilicus with a relaxed abdomen at the end of a gentle expiration. The mean of two measurements was used unless they varied more than 1 cm, then a third measurement was taken, and the two closest measures were used for analysis. WC and height were combined into waist-to-height ratio (WC/Height). Maturity was measured by the Tanner method using an image scale proposed by Carel & Leger (14). Measures were conducted in a private room where each participant was shown pictures of pubic hair and genitalia (both sexes), and breast development for girls. The participants read a brief description for each stage and put a checkmark at the picture that best represented their own development. The room had a safe and relaxed atmosphere and the researchers were the same sex as the participant. Socio-economic status was assessed by questionnaire (15). We used the mothers highest achieved education derived from a question divided by the categories “upper and lower secondary school”, “university <4 years” and “university >4 years”.

Blood pressure was measured by an Omron HBP – 1300 automated monitor (Omron healthcare, inc, Vernon hills, IL, US) after a 10-min rest, in a sitting position without distractions. Measurements were taken in a quiet room on the upper right arm with a size appropriate cuff. Four measurements were taken with 1 minute between each measurement, with the mean of the three last measures being used, unless they varied

more than 5 mmHg, then a fifth measure was taken and the mean of the four last measures being used for analysis.

All blood samples were collected by a nurse or phlebotomist by intravenous blood from the antecubital vein, after an overnight fast, between 08.00 -10.00 AM in the morning. The samples were analyzed for insulin (pmol/L), glucose (mmol/L), triglycerides (mmol/L), total cholesterol (mmol/L), high density lipoprotein (HDL, mmol/L) and low density lipoprotein (LDL, mmol/L) by standard laboratory methods. Both pre and post intervention samples were analyzed at the same time at the accredited endocrine laboratory of the VU medical center (VUmc, Amsterdam, the Netherlands).

Cardiorespiratory fitness was assessed by The Andersen test (16). The test is a 20-m intermittent running test between two parallel lines in a gym hall or similar accommodations. Participants start at one line, run to the other line where they have to touch the floor behind the line with one hand before turning and running back. The test is divided into continuous 15-s periods of running and resting (standing still) separated by a test leaders whistle blow, for a total of 10-min (e.g. 5 minutes of breaks and 5 minutes of running). Participants run as fast as possible and tries to cover the longest distance possible. At the last whistle blow participants stop and their distance is measured and summed as the test result (16). The Andersen test has shown moderate validity (r=0.63) and high reliability (ICC=0.84) for assessing cardiorespiratory fitness on a group level (17,18).

Statistics

We used prospective data from an intervention study and analyzed it as a cohort. The temporal sequence of the metabolic measure makes it possible to determine associations between exposures and outcomes (19). Descriptive characteristics are reported as means with standard deviations and frequencies as percentages. Differences between included/excluded children were tested through independent sample t-tests. Uni- and triaxial differences in the final sample PA estimates were tested through paired sample t-tests, and differences in categorical variables trough Pearson's chi square tests. We computed a cardiometabolic risk score by summing the z-scores of waist-to-height ratio, systolic blood pressure, triglycerides, total cholesterol: high density lipoprotein ratio, The Andersen test (meters) and insulin. We analyzed the prospective associations between sedentary time, LPA, MPA, VPA and MVPA in 1, 10 and 60-s epoch length with the cardiometabolic risk score by multiple linear regression models. The models were adjusted for age, sex, socio economic status, maturity, wear-time and the baseline cardiometabolic risk score. Additionally, sedentary time is adjusted for MVPA and MPA, VPA and MVPA are adjusted for sedentary time. The beta coefficients of the vertical axis were tested against their vector magnitude counterpart in the same epoch length (i.e. sedentary time from the vertical axis in 1-s epoch was compared to the sedentary time form the vector magnitude in 1-s epoch A.S.O). Formal statistical examination of difference in linear regression slopes between the uni- and triaxial estimates were based on the assumption that the standard error of the regression slope was heterogeneous, and each PA estimate had more than 20 observations. We could then use the beta coefficients and the standard error of the regression slopes to calculate differences by the formula: $z = \frac{\beta 1 - \beta 2}{\sqrt{SE1^2 - SE2^2}}$ proposed by Kleinbaum and colleagues

(20). Z-values ± 1.96 represent a statistically significant difference under a conventional 0.05 alpha-level. The dose-response pattern of MVPA in 1, 10 and 60-s epoch length and their association to the cardiometabolic risk score was analyzed through a mixed regression model. The amount of accumulated uni- and triaxial MVPA were divided into quartiles and used as a fixed effect in each of the models respective to their epoch length. The model was adjusted for the same variables as the previous regression models and sedentary time. We used the fourth quartile as reference (highest levels of MVPA accumulated) and school was used as random intercept.

Results

The study sample consisted of 659 children with complete prospective metabolic data, valid accelerometer data and no missing covariables. The anthropometric, demographic and cardiometabolic risk data are shown in table 1. The excluded children were shorter than the included children (mean diff: -1.0 cm [95% CI:-1.920 -0.253] p=0.011), had higher insulin levels (mean: 7.04 pmol/L [95% CI: 1.990 12.097] p=0.006) and performed less LPA (mean: 2.5 min/day [-5.166 -0.013] p=0.049) when measured by uniaxial accelerometry in a 1-s epoch. The excluded children consistently performed less VPA and MVPA both when measured uni- and triaxial and in 1, 10 and 60-s epoch length (all p=<0.05). A description of differences for the included and excluded children is shown in supplementary tables 1 and 2.

Table 1: Anthropometrics, demographics and cardiometabolic risk variables for the final study sample and stratified by sex. The values are expressed as mean and standard deviation unless stated otherwise.

	All participants n=659	Boys n=329	Girls n=330
Anthropometrics			
Age (years)	10.8 (0.2)	10.8 (0.2)	10.8 (0.2)
Height (cm)	147.1 (7.1)	147.1 (7.0)	147.1 (7.3)
Weight (Kg)	39.5 (8.6)	39.2 (8.3)	39.8 (8.9)
Waist: height (ratio)	0.42 (0.04)	0.43 (0.04)	0.42 (0.04)
BMI (Kg/m^2)	18.1 (2.9)	18.0 (2.8)	18.2 (3.0)
Demographics			
Weight status n (%)			
Normal weight*	541 (82.1)	271 (82.4)	270 (81.8)
Overweight	95 (14.4)	47 (14.3)	48 (14.5)
Obese	23 (3.5)	11 (3.3)	12 (3.6)
Pubertal status (Tanner) n (%)			
Stage 1	245 (37.2)	58 (17.6)	187 (56.7)
Stage 2	328 (49.8)	203 (61.7)	125 (37.9)
Stage 3-5	86 (13.0)	68 (20.7)	18 (5.4)
SES (Mothers education) n (%)			
Lower/upper secondary school	253 (38.4)	125 (38.0)	128 (38.8)
University <4 years	195 (29.6)	98 (29.8)	97 (29.4)
University >4 years	211 (32.0)	106 (32.2)	105 (31.8)
Cardiometabolic risk variables			
CRF (Andersen test, meter)	942.5 (97.8)	973.3 (102.3)	911.7 (82.5)
Systolic blood pressure (mmHg)	104.7 (8.0)	104.8 (7.7)	104.5 (8.3)
Diastolic blood pressure (mmHg)	57.5 (5.6)	57.2 (5.7)	57.9 (5.5)
Triglycerides (mmol/l)	0.72 (0.27)	0.68 (0.25)	0.76 (0.27)
Total cholesterol (mmol/l)	4.39 (0.66)	4.39 (0.66)	4.40 (0.65)
HDL cholesterol (mmol/l)	1.63 (0.35)	1.68 (0.35)	1.58 (0.33)
LDL cholesterol (mmol/l)	2.43 (0.60)	2.39 (0.60)	2.46 (0.60)
Total cholesterol: HDL (ratio)	2.78 (0.64)	2.69 (0.59)	2.87 (0.68)
Glucose (mmol/l)	4.95 (0.31)	4.94 (0.32)	4.96 (0.30)
Insulin (pmol/l)	55.80 (29.47)	48.53 (25.18)	63.06 (31.61)

Weight status are based on the age-sex specific reference from Cole et al (13) and pubertal status on Carel & Leger (14). BMI= Body mass index, SES= socio economic status, CRF= cardiorespiratory fitness, HDL= High density lipoprotein, LDL= Low density lipoprotein

*Normal weight includes underweight.

Descriptive PA-levels from uni- and triaxial data reductions at different epoch lengths are presented in table 2. There were significant differences between all uni- and triaxial estimates of sedentary time and PA within the same epoch length (all $p \leq 0.001$). The patterns of sedentary time and PA was similar between the uni- and triaxial estimates and through the epoch length. Longer epoch length led to less time spent sedentary, VPA and MVPA and subsequently more time in LPA. The amount of time spent in MPA did not differ with epoch length and were consistent for both the uni- and triaxial estimates. These differences resulted in the prevalence of children who met the PA recommendations (21) to be significantly higher ($p=<0.05$) when measured triaxially within all epoch lengths and longer epoch lengths resulted in lower compliance. The mean CPM of the vertical axis and vector magnitude were highly correlated ($r=>0.970$) and identical in all epoch lengths (supplementary figure 1).

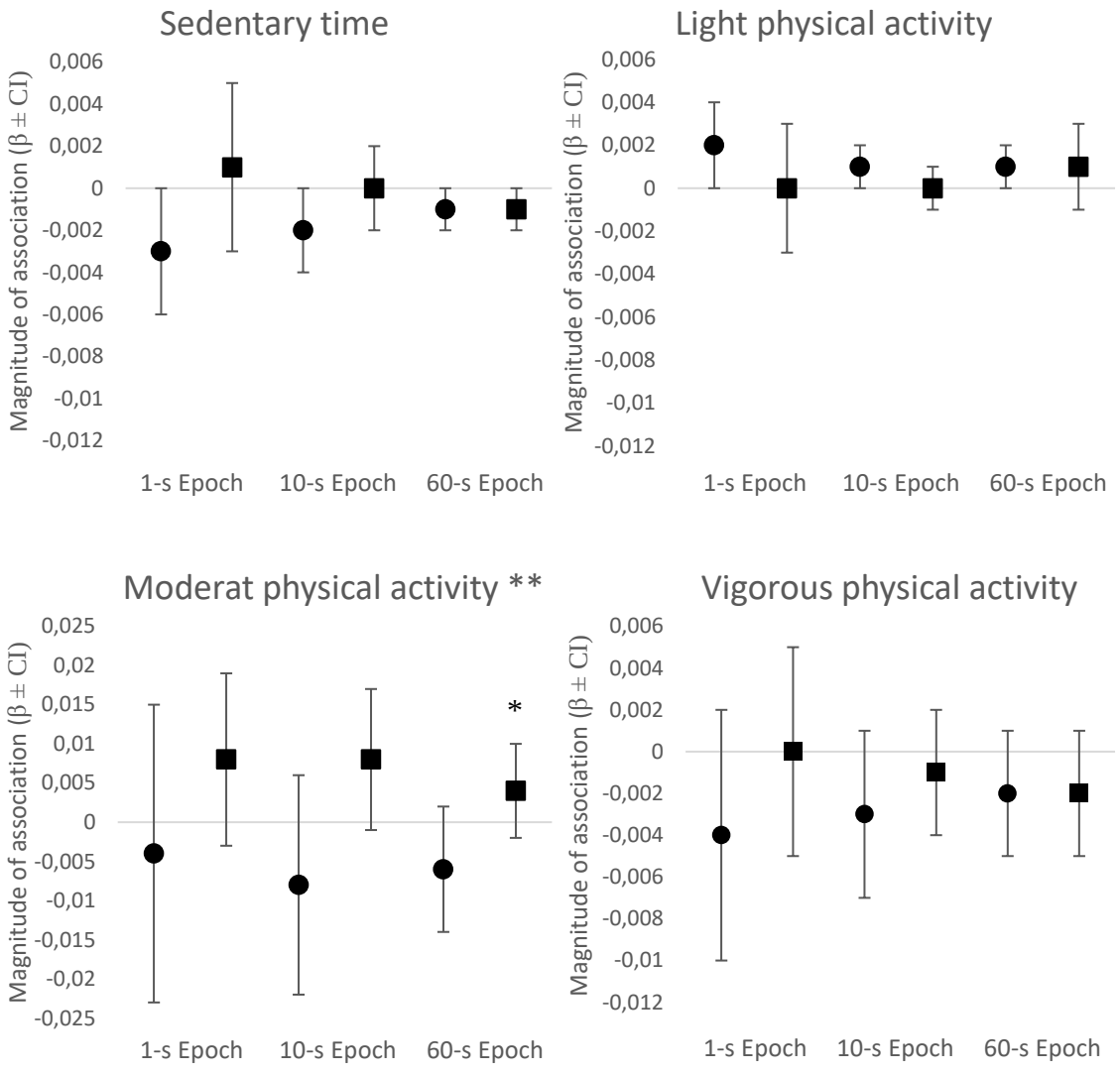
Table 2: Mean and standard deviation of time spent sedentary, light, moderate, vigorous, moderate-to-vigorous physical activity, mean counts per minute, wear-time and valid days for the vertical axis and vector magnitude in different epoch lengths (n=659).

	1-s Epoch	10-s Epoch	60-s Epoch
Vertical axis			
SED (Min)	589.4 (52.9)	504.6 (57.0)	422.8 (59.9)
LPA (Min)	113.9 (20.2)	201.4 (33.2)	290.3 (46.3)
MPA (Min)	21.9 (5.4)	24.5 (6.7)	26.5 (9.7)
VPA (Min)	51.0 (17.4)	45.5 (19.1)	34.6 (20.0)
MVPA (Min)	72.9 (21.7)	70.0 (24.2)	61.2 (27.0)
Mean CPM	694.5 (234.9)	708.0 (252.4)	726.5 (267.6)
Wear-time (Min)	776.4 (50.6)	776.2 (50.7)	774.4 (50.1)
Valid days	6.9 (1.1)	7.0 (1.1)	6.9 (1.1)
Vector Magnitude			
SED (Min)*	521.7 (54.4)	472.6 (58.9)	430.7 (63.3)
LPA (Min)*	122.3 (20.8)	184.3 (30.8)	243.5 (41.2)
MPA (Min)*	52.1 (10.2)	56.3 (12.3)	55.8 (15.8)
VPA (Min)*	80.2 (22.4)	62.9 (23.0)	46.7 (23.6)
MVPA (Min)*	132.4 (30.6)	119.2 (32.7)	102.6 (35.5)
Mean CPM*	1420.8 (361.7)	1355.5 (362.1)	1322.8 (364.9)
Wear-time (Min)	776.7 (51.3)	776.2 (50.7)	777.0 (50.3)
Valid days	6.9 (1.1)	7.0 (1.1)	7.0 (1.1)
Physical activity guideline compliance			
Vertical axis	69.8%	62.8%	46.1%
Vector magnitude**	99.7%	98.2%	88.6%

The PA estimates are based on the uni- and triaxial cut points of Romanzini et al (10). The percentage of children meeting the physical activity recommendations are based on acquiring ≥60 minutes of MVPA per day (21). SED= sedentary time, LPA= light physical activity, MPA= moderate physical activity, VPA= vigorous physical activity, MVPA= moderate to vigorous physical activity, CPM= counts per minute. *= significant difference to the vertical axis estimates (all p=<0.001), **= significant difference to the vertical axis PA guideline compliance (all p=<0.05).

The magnitude of association between uni- and triaxial- sedentary time, LPA, MPA, VPA and MVPA estimates, and the cardiometabolic risk score are shown in figure 2. The magnitude and shape of associations do not seem to follow a general pattern irrespective of epoch length. The patterns in the magnitude of association for sedentary time and VPA were similar. For these intensities, the uniaxial magnitude of association becomes lower when the epoch length increases, while the triaxial estimates becomes greater. The uniaxial estimates of sedentary time and VPA are greater than triaxial in 1 and 10-s epochs, but the magnitude of association is equal over 60-s epoch length. The magnitude of association to LPA was almost identical between the uni- and triaxial estimate and in all epoch lengths. The uniaxial MPA estimates consistently show greater associations to metabolic health in all epoch lengths. The triaxial MPA estimate consistently show lower associations to metabolic health. The difference between the uni- and triaxial estimate in 60-s epoch was statistical significantly different (Table S3). Uniaxial estimates of MVPA show greater association to metabolic health, but to a lesser degree with the increasing epoch length. The triaxial estimates of MVPA were identical inn all epoch lengths.

307 Figure 2: Unstandardized beta coefficients with 95% confidence intervals analyzed by linear
308 regression for the magnitude of association between sedentary time, light, moderate and
309 vigorous physical activity and the cardiometabolic risk score (n=659).



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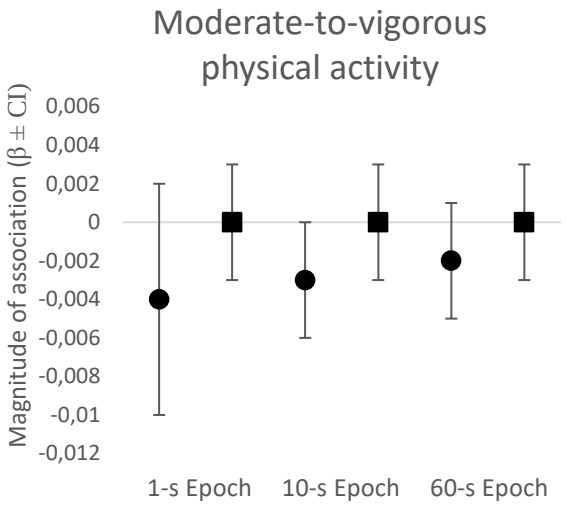
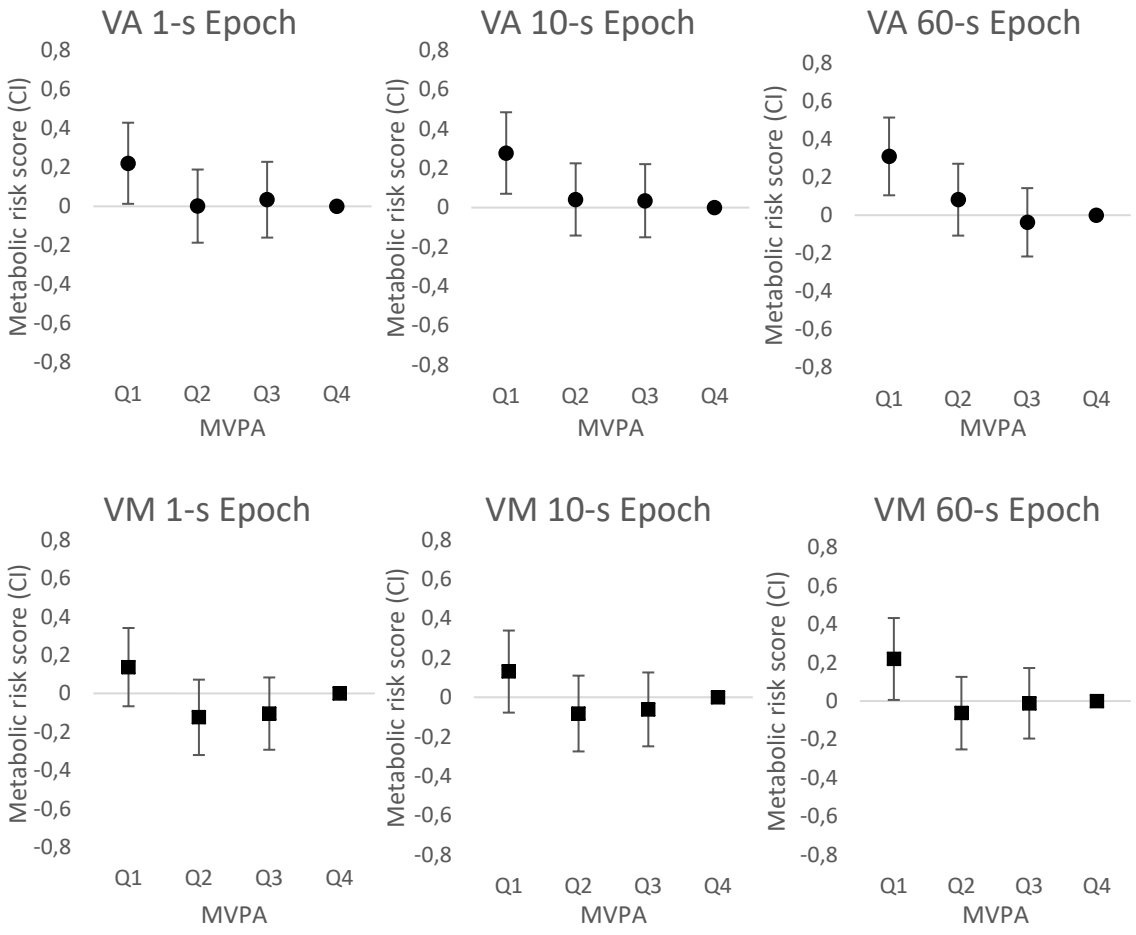


The regression models are controlled for age, sex, maturity, socio economic status, wear-time, and the baseline cardiometabolic risk score. Additionally, sedentary time is controlled for moderate-to-vigorous physical activity and moderate, vigorous and moderate-to-vigorous physical activity is adjusted for sedentary time. β= beta, CI= Confidence intervals, *=significant difference, **=Note: The Y-axis for moderate physical activity differs from the rest. ● = vertical axis, ■ = vector magnitude (n=659).

The pattern of the dose-response association between MVPA and the cardiometabolic risk score were similar between the uni- and triaxial associations regardless of epoch lengths. The first quartile always had the worst association to cardiometabolic health, and there was little difference between the second, third and fourth (reference) quartiles for every epoch length irrespective of the number of axes included. There was little difference within the uni- and triaxial associations when comparing the dose-response patterns across the epoch length. However, there is a consistent greater dose-response association from the least to most active quartile for the uniaxial MVPA measures.

Figure 3: Quartiles of moderate-to-vigorous physical activity analyzed by uni- or triaxial accelerometry with different epoch lengths and their association to the cardiometabolic risk score. The error bars represent the confidence intervals (CI) and the fourth quartile are used as reference. A negative cardiometabolic risk score value means a better risk profile. Each quartile consists of n= 164.



Data were analyzed by mixed regression models adjusted for age, sex, socio economic status, maturity, wear-time, baseline cardiometabolic risk score and sedentary time. School was used as random intercept. The mean minutes of moderate-to-vigorous physical activity in a 10-s epoch for Q1-Q4 derived from the vertical axis were 43.2, 60.1, 75.2 and 98.4 min/day respectively. The mean minutes of moderate-to-vigorous physical activity in a 10-s epoch for Q1-Q4 derived from the vector magnitude were 81.2, 107.8, 127.3 and 156.8 min/day respectively. VA= Vertical axis, VM= Vector magnitude, MVPA= Moderate-to-vigorous physical activity, Q= quartile.

Discussion

This study investigated the impact of common post-processing decisions, uni- versus triaxial data signature and the epoch-length, on associations with a cardiometabolic risk score. Our results show the amount of sedentary time and time spent in PA intensities differ between these data-reduction combinations. The use of longer epoch lengths results in less MVPA. The difference in MVPA results in a large difference in childrens PA guideline compliance. The association patterns to the cardiometabolic risk score were similar within all the uni- and triaxial PA estimates. However, uniaxial estimates of sedentary time, MPA, VPA and MVPA consistently showed greater associations in all epoch lengths. The triaxial estimates of sedentary time, MPA and VPA were positively associated with the cardiometabolic risk score, but when the epoch length increased the association became negative. The uniaxial estimates of sedentary time, MPA and VPA were greater than their triaxial counterparts. The dose-response patterns of MVPA were similar between the uni- and triaxial associations in every epoch length, but the difference between the least and most active quartiles is greater when analyzed uniaxial.

There are very few studies available for comparison to our results. The difference in sedentary time and PA estimates between axes were similar to those reported by Sagelv and colleagues in adults (6). They report that the use of triaxial accelerometry resulted in less sedentary time, more LPA and MVPA compared to the uniaxial estimate in a 10-s epoch. This study's results agree that using triaxial accelerometry yields estimates of less sedentary time and more time spent in MVPA. However, the levels of LPA reported from triaxial data reduction are lower than those derived from the vertical axis only. Although we can only compare the estimates in 10-s epochs, the decrease in

amounts of sedentary time and LPA and the increase in MPA, VPA and MVPA in this study are consistent in all epoch lengths. The changes in our PA estimates as a result of longer epoch lengths follow the same pattern as those described by Banda et al (8). They observed a decrease in sedentary time, VPA and MVPA, an increase in LPA and a stable estimate of MPA when the epoch lengths. While their study offers comparison to the triaxial estimates pattern, they did not include a uniaxial description, so we are unable to directly compare estimate differences. The observed uni- and triaxial differences in our PA estimates in different epoch lengths are in line with earlier research. Considering the short and intermittent PA patterns of children (11), the aggregation of PA in longer epoch lengths could lead short bouted higher intensity PA to be averaged into lower PA intensities if the high-intensity bouts are followed by periods of lower intensity. Similarly, sedentary time could be averaged into higher PA intensities if there are enough higher counts within the epoch length. The required VPA to offset aggregation of sedentary time estimates and sedentary time to offset aggregation of VPA estimates would increase with increasing epoch lengths (22). The decrease in MVPA estimates by increasing epoch lengths is known to affect PA guideline compliance (23). While the uni- and triaxial MVPA estimates and hence PA guideline compliance decreases by increasing epoch lengths, the relative difference in compliance increased (relative difference of 29.9, 35.4 and 42.5% between 1, 10 and 60-s epochs respectively in our sample). These differences are potentially of mayor implications for studies investigating PA guideline compliance over time as the data-reduction methodology needs to be identical if informative trends are to be inferred.

The increasingly higher vector magnitude could be attributed to the offset of the antero-posterior and/or medio-lateral axis (4). This offset would depend on the intensity and triaxial signature of the activity. One study analyzed all the axes separately and as a

vector magnitude and found that the three axes in the vector magnitude showed their strongest association to metabolic health at different intensity intervals. The strongest association to metabolic health for the vertical axis were at 7-7499 CPM, while the antero-posterior axis was found at 6-6499 CPM and the medio-lateral at 100-249 CPM (5). The vector magnitude association pattern reflected the three axes combined and were strongest at 9-10000 CPM. This would suggest that uni- and triaxial PA estimates would be differently associated with metabolic health, not because of the vector magnitude are registering different movement, but because it registers movement differently than the vertical axis alone. Triaxial accelerometry's ability to capture more movement in an acceleration signal (4) could explain some of the observed uni- and triaxial differences. The mathematical implication of combining and squaring the medio-lateral, vertical and antero-posterior axes implies that a triaxial estimate would always be equal to-or greater than a uniaxial estimate. The difference would increase the more movement along the medio-lateral and antero-posterior axis that is recorded (24).

The association patterns of sedentary time, MPA, VPA and MVPA and metabolic health suggest that uniaxial estimates are greater than triaxial in all epoch lengths, especially in 1-s epoch. The PA estimates reflects the calibration protocol for the cut points used. Activities included in protocols are chosen to reflect certain behaviors typical for an intensity. A point of mayor importance that could influence PA classification are the lack of activities to represent LPA and MPA in the Romanzini protocol. When walking/running on a treadmill there is a linear increase in CPM by increased speed up to approximately 10 km/h (25). Despite this the LPA CPM gap (1.5-3 MET=184-2427 CPM) is larger than the MPA gap (3-6 MET=2428-3271 CPM) (10). This seems illogical as the MPA gap should be wider than the LPA. The number -and types of activities selected to represent LPA and MPA should be considered. The LPA

cut points are calibrated only by treadmill walking at 2 km/h. One could argue that more activities are needed to reflect free-living LPA, preferably activity representable of childrens daily activity habits. MPA cut points were calibrated from treadmill walking at 4 km/h and playing volleyball on a court with reduced size. While the MPA cut points includes an activity with a higher triaxial signature, it seems unlikely to represent the majority of childrens daily activities, sport or play. It would be beneficial to include more activities without passive phases and representative of childrens short and intermittent play. There are discrepancies revolving the use of 3 or 4 MET as the cut point for MPA in children. Although 4 MET is preferred (26) this would make the MPA CPM interval in the Romanzini protocol narrower. The result of the lack of activities in the calibration protocol to represent LPA and MPA led to the uniaxial VPA estimate to be higher than the MPA in all epoch lengths. This might be plausible, but seems unlikely, as a pattern of potential misclassification is consistent in every epoch length. The pattern is less evident for the triaxial VPA estimates. The MPA estimates have wider confidence intervals than the other PA intensities in every epoch length. This could limit our ability to accurately draw conclusions about association patterns to cardiometabolic health.

The lack of activities in the calibration protocol to classify LPA and MPA seems to affect the triaxial estimates more than the uniaxial. As previously mentioned, movement along the medio-lateral and antero-posterior axes would be needed to increase the difference between uni- and triaxial PA estimates. It would be beneficial to include more activities in the calibration protocol and especially increase the specificity of the cut points and contribute to a better understanding of uni- and triaxial differences in PA research. Currently an activity with a distinct triaxial signature could vary greatly by intensity, within epoch lengths and in terms potentially be misclassified. Hypothetically,

441 the medio-lateral and antero-posterior axes in the vector magnitude could attenuate
442 associations. However, we know very little as to why this would be the case. It is
443 plausible that triaxial accelerometry captures complex movements better than uniaxial,
444 but at the same time it might be susceptible to picking up more noise in the acceleration
445 signal. The medio-lateral and antero-posterior axis register less movement than the
446 vertical axis. The contribution of the two additional axes are dependent on the triaxial
447 signature of the movement captured. The additional movement captured could provide
448 less health benefits separately than compared to the combined vector magnitude, but
449 this latter is speculative and would need to be confirmed in future studies.

450 The dose-response pattern of MVPA showed the first quartile to consistently have the
451 least favorable cardiometabolic risk. This is not unexpected as those who accumulated
452 more min/day of MVPA are those who show a consistent inverse prospective
453 association to metabolic health (2). However, this is not the case with the triaxial
454 patterns as they show an inverse non-linear pattern where the second and third quartile
455 are considered greater than the reference. The pattern for the second, third and fourth
456 quartile were similar within the uni- and triaxial patterns irrespective of epoch length.
457 Compared to their respective reference, the triaxial second and third quartiles show
458 greater associations. The dose-response patterns of the uniaxial quartiles show slightly
459 stronger associations when comparing the least to most active quartile within all epoch
460 lengths. It seems that the VPA portion of the MVPA estimates describes the dose-
461 response patterns better for the uniaxial patterns, where the triaxial patterns are rather
462 attenuated by the MPA portion, than reflecting the VPA. This could be because there is
463 a lesser percentage contribution from the vertical axis to the vector magnitude estimate
464 for lower intensities. For those who perform the most MVPA it could be related to the
465 context and intensity of the activity, meaning the more MVPA accumulated – a

potentially lower relative contribution of the vertical axis to the vector magnitude. The purposed health benefits from the medio-lateral and antero-posterior axes might influence the associations to a larger degree for those who do not accumulate as much MVPA as those in the reference group. This would be speculative but given the small differences between uni- and triaxial estimates and within triaxial epoch lengths, it might be plausible that some of the MPA accumulated could stem from walking behaviors. Consequently, making the lower quartiles more susceptible for associational shifts due to the portion of MPA in the MVPA estimate possibly having a higher triaxial signature.

In this study we have shown that there are considerable differences between uni- and triaxial accelerometer derived PA estimates. While this is not unexpected, we uncovered differences in associations to cardiometabolic health markers while the dose-response patterns for MVPA derived using different post-processing criteria were similar. These differences are diminished by summation of PA in longer epoch lengths. The influence of epoch lengths could be explained by an activity's intensity and its degree of triaxial signature. PA research is at a crossroad where the traditional uniaxial approach is challenged by triaxial accelerometry and we need to know how triaxial accelerometry compares to earlier results. This study's result indicate that there are differences in the magnitude of associations between uni- and triaxial derived sedentary time, LPA, MPA, VPA and MVPA in different epoch lengths. This difference is most apparent in the shortest epoch length. When using the post-processing decisions in this study, the reason for the uni- and triaxial difference in magnitude of association remains unclear. Triaxial accelerometry might have a future use for capturing complex agile movement compared to previous uniaxial data-reduction. More research is needed to uncover if

uni- and triaxial differences are due to post-processing decisions or the triaxial signature of the recorded activities.

The main implication of our study is that the use of triaxial accelerometry leads to different magnitude of associations but similar shapes of the dose-response patterns in different epoch lengths, compared to uniaxial accelerometry. These findings suggest these post-processing decisions can lead researchers to different conclusions in some aspects of the role physical activity in promoting cardiometabolic risk markers in children.

Strength and limitations

To our knowledge this is the first study to compare uni- and triaxial accelerometer derived PA and its association to childrens prospective cardiometabolic health. In contrast to earlier studies we can estimate potential benefits of altered PA levels and the association to cardiometabolic health markers. We applied a set of cut points applicable to our sample where the uni- and triaxial cut points were calibrated from the same data.

Our study has some limitations. Although our cut points were calibrated from the same data, the calibration protocol may lack activities to sufficiently reflect free-living LPA and MPA. The activities included in the protocol should have included a higher triaxial signature to increase accuracy for the triaxial cut points. While sedentary time and VPA seems to be accurately reflected, uncertainty in the differentiating between LPA and MPA should be noticed. If the future of PA registration is triaxial, cut points should be calibrated from the same data consisting of more activities with a higher triaxial signature- preferably agile sports and other daily activities typical to the study population and PA intensities of interest.

Our study sample are all the same age and from the same county. Maturation has the potential to limit our results generalizability due to biomechanical differences between children, adolescents and adults. Lower extremity length is regarded as a key determinant for stride length and would result in differences in step frequencies and hence differences in internal and external moment creating more noise in the acceleration signal for children (24). The amount of noise contributing to the movement signal could be larger when measured triaxial, but this is unknown. The geographical landscape of the county differs from bigger cities in terms of infrastructure and modes of transportation. Accelerometers ability to capture activities with non-ambulatory movement at the hip, such as cycling, weightbearing activities and inclined walking, such as walking to school with a backpack, could have contributed to some misclassification for children with active transport to/from school (27).

Conclusion

Triaxial physical activity estimates show lower prospective associations to cardiometabolic health, especially for moderate, vigorous and moderate-to-vigorous physical activity. The uni- and triaxial difference in association is attenuated in longer epoch lengths. The dose-response patterns of MVPA are similar between the number of axes used and epoch length, but the magnitude of association between the least and most active quartile are greater when measured uniaxial.

References

1. Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput JP, Janssen J, Katzmarzyk PT. et al. Systematic review of the relationship between objectively measured physical activity and health indicators in school-aged children and youth. Appl Physiol Nutr Metab. 2016;41:197-239.
2. 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 faktors in youth: a systematic review and meta-analysis. Obes Rev. 2019;20:55-74.
3. Wijndaele K, Westgate K, Stephens SK, Blair SN, Bull FC, Chastin SFM. et al. Utilization and harmonization of adult accelerometry data: Review and expert consensus. Med Sci Sports Exerc. 2015;47(10):2129-39.
4. Smith MS, Horsch A, Standl M, Heinrich J, Schulz H. Uni- and triaxial accelerometric signals agree during daily routine, but show differences between sports Sci Rep. 2018;8(15055).
5. Aadland E, Kvalheim OM, Anderssen SA, Resaland GK, Andersen LB. The triaxial physical activity signature associated with metabolic health in children. Med Sci Sports Exerc. 2019;51(10).
6. Sagelv EH, Ekelund U, Pedersen S, Brage S, Hansen BH, Johansson J. et al. Physical activity levels in adults and elderly from triaxial and uniaxial accelerometry. The Tromsø study. PLoS ONE. 2019;14(12):e0225670.
7. Chomistek AK, Yuan C, Matthews CE, Troiano RP, Bowles HR, Rood J. et al. Physical activity assessment with the actigraph GT3X and doubly labeled water. Med Sci Sports Exerc. 2017;49(9):1935-44.
8. Banda JA, Haydel KF, Davila T, Desai M, Bryson S, Haskell WL. et al. Effects of varying epoch lengths, wear time algorithms, and activity cut-point on estimates of children sedentary behavior and physical activity from accelerometer data. PLoS ONE. 2016;11(3):e0150534.
9. Resaland GK, Moe VF, Aadland E, Steene-Johannessen J, Glosvik Ø, Andersen JR. et al. Active smarter kids (ASK): rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on childrens academic performances and risk factors for non-communicable diseases. BMC Public Health. 2015;15(709).
10. Romanzini M, Petroski EL, Ohara D, Dourado AC, Reichert FF. Calibration of actigraph GT3X, actical and RT3 accelerometers in adolescents Eur J Sports Sci. 2014;14(1):91-9.
11. Brooke HL, Atkin AJ, Corder K, Brage S, van Sluijs EMF. Frequency and duration of physical activity bouts in school-aged children: A comparison within and between days. Prev Med Rep. 2016;4:585-90.
12. Møller NC, Tarp J, Kamelarczyk EF, Brønd JC, Klakk H, Wedderkopp N. Do extra compulsory physical education lessons mean more physically active children – findings from the childhood health, activity, and motor performance school study Denmark (The CHAMPS-study DK). Int J Behav Nutr Phys Act. 2014;11(121).
13. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. BMJ. 2000;320:1-6.
14. Carel JC, Leger J. Precocious puberty. N Engl J Med. 2008;258:2366-77.
15. The Norwegian directorate of health. Physical activity among 6, 9 and 15 year olds. Results from surveillance in 2011. IS-2002, 06/2012. Oslo.

16. Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. J Sport Med Phys Fit. 2008;48(4):434-37.
17. 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(10):e110492.
18. Batista MB, Romanzini CLP, Castro-Pinero J, Vaz Ronque ER. Validity of field tests to estimate cardiorespiratory fitness in children and adolescents: a systematic review. Rev Paul Pediatr. 2017;35(2):222-33.
19. Bennett DA. Review of analytical methods for prospective cohort studies using time to event data: single studies and implications for meta-analysis. Stat Methods Med Res. 2003;12:297-319.
20. Kleinbaum DG, Kupper LL, Nizam A, Rosenberg ES. Applied regression analysis and other multivariable methods. 5th ed. Boston: Cengage Learning.
21. Anbefalinger om kosthold, ernæring og fysisk aktivitet. (2014). (IS-2170). Oslo: Helsedirektoratet.
22. Aadland E, Andersen LB, Anderssen SA, Resaland GK, Kvalheim OM. Accelerometer epoch setting is decisive for associations between physical activity and metabolic health in children. J Sports Sci. 2019;38(3):256-63.
23. Aibar A, Bois JE, Zaragoza J, Generelo E, Julian JA, Paillard T. Do epoch lengths affect adolescents compliance with physical activity guidelines? J Sports Med Phys Fitness. 2014;54:326-34.
24. Fridolfsson J, Börjesson M, Arvidsson DA. A biomechanical re-examination of physical activity measurements with accelerometers Sensors. 2018;18(3399).
25. John D, Miller R, Kozey-Keadle S, Caldwell G, Freedson P. Biomechanical examniation of the “plateau phenomenon” in ActiGraph vertical activity counts. Physiol Meas. 2012;33(2):219-30.
26. 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(7):1360-68.
27. Lee IM, Shiroma E. Using accelerometers to measure physical activity in large-scale epidemiologic studies: Issues and challenges Br J Sports Med. 2014;48(3):197-201.

Supplementary files

Table S1: Independent sample t-tests for the difference in baseline anthropometry and cardiometabolic risk variables between the included and excluded children at baseline (n=912). The number of excluded children (n=>305) are those who miss one or more of the variables at either baseline or post intervention.

	p= between groups	Mean difference	95% CI Lower	Upper
Age (years n=432)	0.877	0.002	-0.032	0.037
BMI (Kg/m^2 n=398)	0.576	0.105	-0.263	0.473
Weight (Kg n=398)	0.475	-0.362	-1.358	0.633
Height (cm n=399)	**0.011**	**-1.086**	**-1.920**	**-0.253**
WThR (ratio n=397)	0.157	0.004	-0.001	0.009
Andersen (meter n=348)	**<0.001**	**-30.220**	**-43.376**	**-17.064**
SBP (mmHg n=389)	0.728	-0.186	-1.236	0.863
DBP (mmHg n=389)	0.214	0.489	-0.282	1.262
Cholesterol (mmol/l n=307)	0.855	0.008	-0.084	0.101
HDL (mmol/l n=307)	0.409	-0.019	-0.065	0.026
TC:HDL Ratio (ratio n=307)	0.444	0.036	-0.057	0.131
LDL (mmol/l n=306)	0.746	0.014	-0.071	0.100
Triglycerides (mmol/l n=307)	0.148	0.036	-0.012	0.084
Glucose (mmol/l n=307)	0.111	0.038	-0.008	0.086
Insulin (pmol/l n=305)	**0.006**	**7.043**	**1.990**	**12.097**

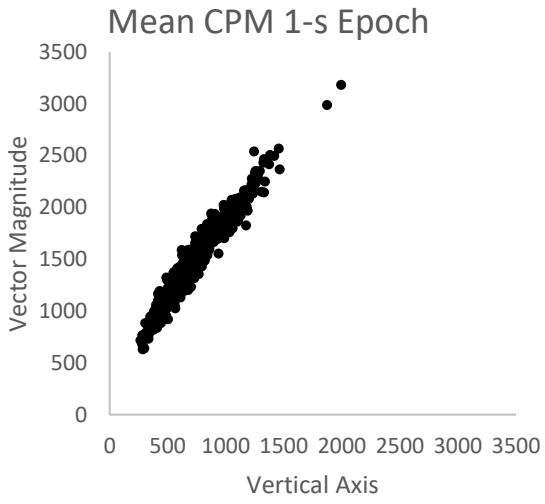
CI= Confidence interval, BMI= Body mass index, WThR= Waist-to-height ratio, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, HDL= High density lipoprotein, TC:HDL ratio= Total cholesterol: High density lipoprotein ratio, LDL= Low density lipoprotein. Bold entries represent statistical differences.

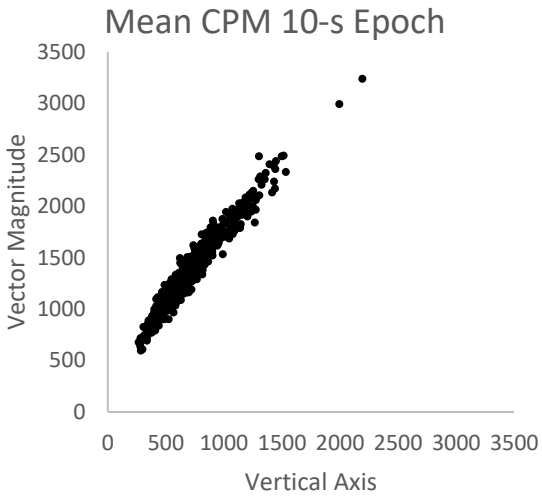
Table S2: Paired sample t-tests for the difference between the included and excluded childrens sedentary time, light, moderate, vigorous and moderate-to-vigorous physical activity at baseline (n=1075). The number of excluded children are those who are missing prospective cardiometabolic, covariate or accelerometer data (n=>373).

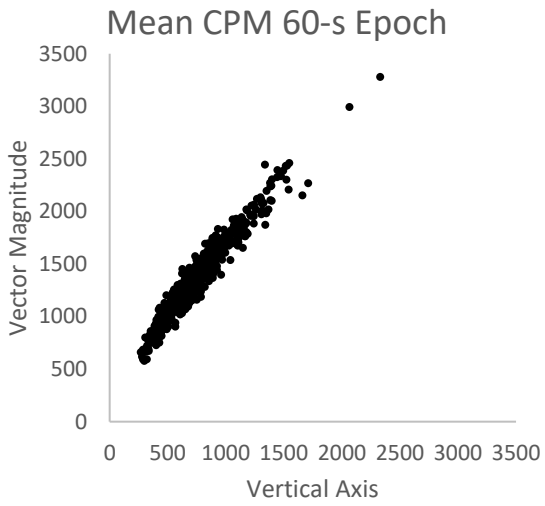
	p= between groups	Mean difference	95% CI Lower	Upper
Vertical axis				
1-s Epoch (374)				
SED	0.570	1.990	-4.882	8.863
LPA	**0.049**	**2.589**	**-5.166**	**-0.013**
MPA	0.338	-0.349	-1.065	0.366
VPA	**0.001**	**-3.719**	**-5.899**	**-1.539**
MVPA	**0.004**	**-4.069**	**-6.806**	**-1.334**
10-s Epoch (375)				
SED	0.314	3.766	-3.575	11.109
LPA	0.091	-3.646	-7.873	0.580
MPA	0.283	-0.496	-1.403	0.410
VPA	**0.001**	**-4.078**	**-6.456**	**-1.700**
MVPA	**0.003**	**-4.574**	**-7.632**	**-1.517**
60-s Epoch (373)				
SED	0.246	4.583	-3.162	12.328
LPA	0.073	-5.446	-11.398	0.506
MPA	0.141	-0.948	-2.212	0.315
VPA	**0.001**	**-4.041**	**-6.501**	**-1.582**
MVPA	**0.004**	**-4.990**	**-8.383**	**-1.596**
Vector magnitude				
1-s Epoch (374)				
SED	0.419	2.944	-4.207	10.097
LPA	**0.033**	**-2.903**	**-5.569**	**-0.236**
MPA	0.471	-0.495	-1.844	0.852
VPA	**0.004**	**-4.214**	**-7.052**	**-1.377**
MVPA	**0.019**	**-4.710**	**-8.638**	**-0.782**
10-s Epoch (375)				
SED	0.333	3.796	-3.892	11.485
LPA	0.072	-3.604	-7.536	0.327
MPA	0.576	-0.477	-2.154	1.198
VPA	**0.005**	**-4.168**	**-7.074**	**1.262**
MVPA	**0.030**	**-4.646**	**-8.838**	**-0.435**
60-s Epoch (378)				
SED	0.270	4.629	-3.600	12.858
LPA	0.101	-4.429	-9.723	0.865
MPA	0.316	-1.055	-3.119	1.007
VPA	**0.005**	**-4.274**	**-7.234**	**-1.314**
MVPA	**0.022**	**-5.330**	**-9.872**	**-0.787**

CI= Confidence intervals, SED= sedentary behavior, LPA= Light physical activity, MPA= moderate physical activity, VPA= vigorous physical activity, MVPA= moderate-to-vigorous physical activity. Bold entries represent statistical differences.

Figure S1: Correlation plot of the mean counts per minute between the vertical axis and vector magnitude in 1, 10 and 60-s epoch length (n=659).







CPM= counts per minute

Table S3: Beta coefficients, standard errors of the vertical axis and vector magnitude estimates in different epoch settings used for calculating differences in regression slopes. Bold entries represent a statistically significant difference (n=659).

		Beta coefficient (SE)		$\frac{\beta 1 - \beta 2}{\sqrt{SE1^2 - SE2^2}}$
		VA	VM	
1-s Epoch	SED	-0.003 (0.002)	0.001 (0.002)	-1.41
	LPA	0.002 (0.001)	0.000 (0.001)	1.41
	MPA	-0.004 (0.010)	0.008 (0.006)	-1.03
	VPA	-0.004 (0.003)	0.000 (0.002)	-1.11
	MVPA	-0.004 (0.003)	0.002 (0.002)	-1.66
10-s Epoch	SED	-0.002 (0.001)	0.000 (0.001)	-1.41
	LPA	0.001 (0.001)	0.000 (0.001)	0.71
	MPA	-0.008 (0.007)	0.008 (0.005)	-1.86
	VPA	-0.003 (0.002)	-0.001 (0.002)	-0.71
	MVPA	-0.003 (0.002)	0.000 (0.002)	-1.06
60-s Epoch	SED	-0.001 (0.001)	-0.001 (0.001)	0.00
	LPA	0.001 (0.001)	0.001 (0.001)	0.00
	MPA	**-0.006 (0.004)**	**0.004 (0.003)**	**-2.00**
	VPA	-0.002 (0.002)	-0.002 (0.002)	0.00
	MVPA	-0.002 (0.002)	0.000 (0.001)	-1.41

SE= Standard Error, β= Beta, VA= Vertical axis, VM= Vector magnitude, SED= Sedentary behavior, LPA= Light physical activity, MPA= Moderate physical activity, VPA= Vigorous physical activity, MVPA= moderate-to-vigorous physical activity. Bold entries represent a statistical difference.