



Statistical advancements in analyzing accelerometer-measured physical activity intensity

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GOTHENBURG

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Abstract

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Physical activity (PA) is widely recognized as an important factor in preventing and treating cardiometabolic diseases and reducing mortality. Yet, the health implications of specific PA intensities and the intricate role of fitness in the relationship between PA and health remain less clear. While accelerometers provide objective measurements of PA intensity, established methods for data processing and statistical analysis often underutilize this information. Recent advancements in accelerometer data processing and multivariate statistical methods promise enhanced detailed analyses of PA intensity. This doctoral thesis aimed to introduce and further develop multivariate statistical methods to analyze accelerometer-measured PA intensity.

Data previously collected from four separate studies were re-analyzed using improved accelerometer data processing methods and multivariate statistical approaches. Specifically, data from the LIV 2013, SCAPIS, I.Family, and Bunkeflo studies were included. The improved accelerometer data processing method employed a 10 Hz frequency filter, instead of the common 1.63 Hz filter, facilitating the capture of moderate-to-vigorous intensity PA. All the multivariate statistical techniques employed were based on partial least squares regression (PLS). PLS was applied to explore the association between PA intensity and health. Extensions of the PLS model, including PLS discriminant analysis and PLS structural equation modeling, were used for group comparisons and mediation analysis, respectively.

The results highlight the importance of detailed analyses of PA intensity. Using a wider frequency filter in the processing of raw accelerometer data resulted in stronger associations with health indicators and allowed for a more detailed interpretation of PA intensity. The patterns of PA intensity relating to health were different for different health indicators and different groups. Fitness level determined the PA intensity required for associations with health and can be considered an indicator of sufficient PA for health benefits. Analysis of PA patterns using multivariate statistical methods captures more detail in the accelerometer data and enables studying the complex role of PA intensity in different study designs.

Svensk sammanfattning

Fysisk aktivitet är allmänt känt som en viktig faktor för att förebygga och behandla kardiometabola sjukdomar och minska mortalitet. Trots det är hälsokonsekvenserna av fysisk aktivitet på specifika intensitetsnivåer och den komplexa rollen av kondition i förhållandet mellan fysisk aktivitet och hälsa fortfarande mindre tydliga. Det har blivit vanligt att använda accelerometrar i forskning för att mäta fysisk aktivitet, men metoderna för databearbetning och statistisk analys använder bara en bråkdel av den information som samlas in från accelerometrar. Nya framsteg inom bearbetning av accelerometerdata och multivariata statistiska metoder skulle kunna användas för mer detaljerade analyser av fysisk aktivitetsintensitet. Denna doktorsavhandlingens syfte var att introducera och vidareutveckla multivariata statistiska metoder för analys av fysisk aktivitetsintensitet uppmätt med accelerometrar.

Tidigare insamlade data från fyra separata studier analyserades på nytt med hjälp av förbättrade metoder för bearbetning av accelerometerdata och multivariat statistik. Data från studierna LIV 2013, SCAPIS, I.Family och Bunkeflo inkluderades. Den förbättrade metoden för accelerometerdatabearbetning använde ett frekvensfilter på 10 Hz i stället för det vanligaste förekommande 1.63 Hz-filtret, vilket fångar fysisk aktivitet på måttlig till hög intensitet bättre. De multivariata statistiska metoderna som användes baserades på Partial Least Squares regression (PLS). PLS användes för att undersöka sambandet mellan fysisk aktivitetsintensitet och hälsa. Tillägg till PLS-modellen användes också i form av PLS diskriminantanalys för gruppjämförelser och PLS strukturell ekvationsmodellering för medieringsanalys.

Resultaten framhåller vikten av detaljerade analyser av fysisk aktivitetsintensitet. Att använda ett vidare frekvensfilter i bearbetningen av accelerometerdata resulterade i starkare samband med hälsoindikatorer och möjliggjorde en mer detaljerad tolkning av fysisk aktivitetsintensitet. De mönster av fysisk aktivitet som visade vilken intensitet som var starkast kopplad till hälsa var olika för olika hälsoutfall och för olika grupper. Individens konditionsnivå avgjorde vilken fysisk aktivitetsintensitet som var tillräcklig för hälsovinster och kan betraktas som en indikator på tillräcklig fysisk aktivitet för hälsofördelar. Analys av fysisk aktivitetsmönster med hjälp av multivariata statistiska metoder fångar mer detaljer i accelerometerdata och gör det möjligt att undersöka den komplexa rollen för fysisk aktivitetsintensitet i olika studiedesigner.

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List of original papers

This thesis is based on the following papers, referred to in the text by their Roman numerals.

- I. Jonatan Fridolfsson, Mats Börjesson, Elin Ekblom-Bak, Örjan Ekblom, Daniel Arvidsson. **Stronger Association between High Intensity Physical Activity and Cardiometabolic Health with Improved Assessment of the Full Intensity Range Using Accelerometry.** *Sensors*. 2020 Feb 18;20(4):1118.
- II. Jonatan Fridolfsson, Christoph Buck, Monica Hunsberger, Joanna Baran, Fabio Lauria, Denes Molnar, Luis A. Moreno, Mats Börjesson, Lauren Lissner, Daniel Arvidsson, on behalf of the I.Family consortium. **High-intensity activity is more strongly associated with metabolic health in children compared to sedentary time: a cross-sectional study of the I.Family cohort.** *International Journal of Behavioral Nutrition and Physical Activity*. 2021 Jul 6;18(1):90.
- III. Jonatan Fridolfsson, Daniel Arvidsson, Lars Bo Andersen, Ola Thorsson, Per Wollmer, Björn Rosengren, Magnus K. Karlsson, Magnus Dencker. **Physical activity spectrum discriminant analysis – A method to compare detailed patterns between groups.** *Scandinavian Journal of Medicine & Science in Sports*. 2021 Sep 18;31(12):2333–42.
- IV. Jonatan Fridolfsson, Daniel Arvidsson, Elin Ekblom-Bak, Örjan Ekblom, Göran Bergström, Mats Börjesson. **Accelerometer-measured absolute versus relative physical activity intensity: cross-sectional associations with cardiometabolic health in midlife.** *BMC Public Health*. 2023 Nov 24;23(1):2322.
- V. Jonatan Fridolfsson, Elin Ekblom-Bak, Örjan Ekblom, Göran Bergström, Daniel Arvidsson, Mats Börjesson. **Fitness-related physical activity explains most of the association between accelerometer data and cardiometabolic health in 50-64 years old.** In manuscript.

Abbreviations

| | | |
|----------|-------|----------------------------------------------------|
| BMI | | Body mass index |
| CS | | Cardiometabolic risk factor composite score |
| DLW | | Doubly labeled water |
| <i>g</i> | | Gravity, 9.82 m/s ² |
| HbA1c | | Glycated hemoglobin |
| HDL | | High-density lipoprotein |
| HOMA | | Homeostasis model assessment |
| LDL | | Low-density lipoprotein |
| METs | | Metabolic equivalents of task |
| mg | | Milli-gravity, <i>g</i> /1000 |
| MVPA | | Moderate-to-vigorous physical activity |
| PA | | Physical activity |
| PLS | | Partial least squares regression |
| PLS-DA | | Partial least squares discriminant analysis |
| PLS-SEM | | Partial least squares structural equation modeling |
| SBP | | Systolic blood pressure |
| SCAPIS | | Swedish CARDioPulmonary BioImage Study |
| WHO | | World Health Organization |

Background

Physical activity

Definition

Physical activity (PA) is defined as “any bodily movement produced by skeletal muscles that results in energy expenditure” (1). This implies that PA is related to both the physical active movement of the body (-parts) and the physiological energy expenditure that comes with actively contracting skeletal muscles. PA intensity is typically quantified as multiples of resting energy expenditure referred to as metabolic equivalents of task (METs) (2). Furthermore, PA intensity is divided into light, moderate, vigorous, and very vigorous defined as METs above 1.5, 3, 6, and 9 respectively. PA below 1.5 METs is considered sedentary. To facilitate interpretation, moderate PA is considered equivalent to brisk walking, and vigorous PA is equivalent to running. PA intensity only refers to energy expenditure and not activity type, e.g. sedentary includes sitting, standing, and lying down (3).

Health benefits

The primary reason for the overall interest in PA is its relation to health. It is considered a main factor for reducing mortality (4,5), and is increasingly being considered a “vital sign” in clinical practice (6). Moreover, the importance of PA for physical functioning, mental health, and quality of life is well established (7). Specifically, the relationship between PA and cardiometabolic health is strong, where increased PA is associated with a lower risk of cardiometabolic disease (5). One-third of the population worldwide is considered insufficiently physically active based on self-report (8), which is estimated to be the cause of around one-third of cardiometabolic disease globally (9).

Cardiometabolic disease refers to cardiovascular disease, diabetes mellitus, and chronic renal failure (10). Cardiovascular disease in turn includes a range of disorders where a majority of the morbidity is caused by ischemic heart disease and stroke (11). Cardiometabolic disease displays similar risk factors, namely

obesity, hypertension, insulin resistance, and dyslipidemia (11,12). These risk factors are considered early signs of cardiometabolic disease and can predict future cardiometabolic disease to a large degree (13). Although these risk factors are more prevalent in adults, they are also apparent in children and adolescents (14–16). Further, individuals with risk factors at a young age are more likely to have poor metabolic health later in life (17,18). PA displays favorable associations with all of the cardiometabolic risk factors above, which in turn reduce the risk of cardiometabolic disease (2).

Similar to PA, cardiorespiratory fitness, defined as maximal oxygen uptake, is a strong predictor for mortality and cardiometabolic disease (19,20). It is often used synonymously with PA (21), since the two are highly correlated with each other in cross-sectional studies, and increased PA increases fitness in intervention studies (2). However, it could be problematic to use the terms synonymously. Fitness could both be considered a mediator and moderator in the relationship between PA and health where additionally both PA and fitness are independently associated with health (2).

The cellular and molecular mechanisms by which PA improves health are not fully understood. PA increases metabolism and leads to changes in various tissues, particularly in skeletal muscles. These changes are mainly due to individual bouts of PA which in turn result in alterations in muscle cells, enhancing their capacity and releasing myokines, extracellular vesicles, and metabolites, which in turn have a positive impact on other tissues (22). Some health benefits are thought to mainly be related to the overall increase in energy expenditure from PA, whereas other health benefits likely require PA of sufficient intensity (23).

In general, PA of at least moderate intensity seems to be required to achieve most of the health benefits. However, conflicting results suggest that light intensity could be sufficient, whereas other results suggest vigorous intensity is required. Some of the controversies regarding the health benefits of different PA intensities can be explained by the health-beneficial PA intensity being relative to individual fitness level. Hence, PA at absolute moderate intensity (3 METs) does not have the same effect on health outcomes for everyone. Instead, definitions of PA intensity relative to fitness level are above 46, 64, and 91% of maximal oxygen uptake for moderate, vigorous, and very vigorous intensity, respectively. For example, for an older individual with low fitness, walking slowly could be sufficient to achieve most health benefits of PA, whereas a younger individual with high fitness may require brisk walking, or even running, to achieve most health benefits. In addition, vigorous intensity PA might have additional health benefits for all

individuals, but adherence to vigorous intensity PA might be lower compared to moderate intensity and is not suitable for individuals with some medical conditions. (23)

Recommendations on physical activity

The relationship between PA and cardiometabolic disease gained increased attention in 1953 when Jeremy N Morris published a paper about the risk of cardiometabolic disease among drivers and conductors of London buses (24). He found that the drivers had an increased risk of cardiometabolic disease compared to the conductors and argued that this was because the drivers were sitting all day whereas the conductors were constantly walking around the busses, climbing up and down the stairs. This work was followed by studies investigating this association among other occupational groups in an attempt to show that this relationship was independent of work environment and socioeconomic status (25). Morris used statistical methods from the field of medicine to analyze these associations and by this, connected the field of PA research with epidemiology. With time, studies about the health benefits of PA became more extensive. One example is Steven N. Blair who found a strong relationship between fitness and mortality among over 13 000 individuals that were followed for 8 years (26).

The first general recommendations on PA were launched in 1996 by the American Surgeon General (27). These recommendations stated that the general public should engage in at least 30-45 minutes of PA most days of the week to decrease their risk of cardiometabolic disease. This should be performed as brisk walks, cycling, gardening, etc., which would be considered PA of moderate intensity. PA was recognized as a part of health promotion by the World Health Organization (WHO) in 2004 when they released their “Global Strategy on Diet, Physical Activity and Health” suggesting that all nations should develop their recommendations on PA (28). In 2010 WHO released its recommendations on PA suggesting 150 minutes of moderate intensity PA per week, performed in bouts of at least 10 minutes (29). Practically, these recommendations are very similar because 30 minutes 5 times a week equals 150 minutes. In addition, both the bout requirement and the requirement of performing PA most days of the week make it slightly more difficult to reach the recommendations, but in different ways (30). Furthermore, the WHO guidelines also stated that the 150 minutes of moderate PA could be replaced by 75 minutes of vigorous PA. Alternatively a combination

of the two where 1 minute of vigorous PA would be equivalent to 2 minutes of moderate PA.

More recently, the PA recommendations from WHO were updated (7,31). Most of the new recommendations were the same as previously but with three clear distinctions. First, the requirement of accumulating PA in bouts of 10 minutes was discarded. This was motivated by new research mainly based on objective measurement that suggested that also PA in short bouts was beneficial for health (2). Second, the recommendations also say that 300 minutes of moderate PA per week is associated with more health benefits. Third, the guidelines also included recommendations to limit sedentary time (7,31). It was also emphasized that especially individuals with a lot of sedentary time were encouraged to engage in at least 300 minutes of PA.

Sweden followed the initial WHO recommendations of 150 minutes per week but has in some settings been interpreted more strictly as 30 days at least 5 days a week (30,32). After the revision of the WHO recommendations, Sweden updated their recommendations accordingly but did so by the public health agency of Sweden officially releasing their recommendations (33). In addition, more individually tailored recommendations on PA have been developed for clinical settings through FYSS (Swedish abbreviation of physical activity in prevention and treatment of disease), which include PA on prescription (34). The PA on prescription model developed and used in Sweden is plausibly the most successful worldwide, consisting of patient-centered dialogue, individually adapted recommendations, and follow-up (35).

The 2010 PA guidelines from WHO also included recommendations for children and adolescents. These recommendations suggest at least 60 minutes of moderate-to-vigorous physical activity (MVPA) per day including vigorous PA at least three times a week (29). The 2020 updated guidelines on PA for children and adolescents also included recommendations to limit sedentary time similar to the new recommendations for adults (7,31).

A few problems are apparent with the development of the current recommendations on PA. First, the recommendations are expressed in relative terms, such as moderate intensity, whereas accelerometer-measured PA is expressed in absolute terms such as METs (2). For inactive individuals lower intensity is sufficient to improve health, much lower than the typical 3 METs (36). Therefore, PA intensity level should ideally be expressed relative to individual fitness level. Second, the 150 minutes per week cut-off was initially set because of studies suggesting that this was the lowest level where statistically significant health

improvements could be observed (37). More recent research with higher quality shows that health benefits are apparent at much lower PA volumes and that volumes much higher than 150 minutes per week are of additional benefit (2). Third, the initial recommendations were solely based on self-reported data (29,37,38). Modern objective measurements of PA could give data of much higher quality and detail about the relationship between PA and health (39).

Measurement of physical activity

Measurement of PA is important in health promotion and medicine to provide a foundation for guidelines as well as evaluate the effects of interventions (39,40). The measurement is usually focused either on the physical part of PA, actively moving the body, or on the physiological part, energy expenditure. Furthermore, measurement methods consist of either subjective or objective methods.

Subjective methods are for example: questionnaires, interviews, and diaries. The subjective methods are prone to measurement errors caused by misinterpretation, social desirability, and memory limitations (39). In general, individuals over-report their level of PA with subjective methods compared to objectively measured PA by accelerometer (41). More importantly, stronger associations are seen with accelerometer-measured PA and markers of cardiometabolic health compared to self-reported PA (42,43).

The gold standard for measuring PA in a lab setting is to use indirect calorimetry for the physiological aspect of PA which is energy expenditure (44), or force plates and 3D movement tracking systems for the physical aspect of PA which is mechanical work (45). However, these methods are not applicable for prolonged measurements in free-living settings, which are required to capture habitual PA.

The doubly labeled water (DLW) method is considered the gold standard for prolonged measurement of energy expenditure in a free-living setting. DLW consists of enriched heavy hydrogen (^2H) and heavy oxygen (^{18}O). To measure energy expenditure, subjects consume a specified amount of DLW followed by a collection of urine samples over 14 days. Urine samples are analyzed by a mass spectrometer to establish the rate of oxygen and hydrogen elimination. From the difference between the two elimination rates, it is possible to calculate the carbon dioxide production, which in turn can be used to estimate energy expenditure accurately. The major downside of DLW is the cost of materials and analyses as well as the logistics related to collecting urine samples. In addition, DLW only

provides a measurement of total energy expenditure over the measurement period and is unable to differentiate between high volumes of light intensity PA and low volumes of vigorous-intensity PA. (46)

Energy expenditure can also be estimated in free-living settings by heart rate measurements. Since individual heart rate variation is high, these measurements are only able to provide accurate measurements of energy expenditure if individually calibrated (47). Individual calibration of heart rate is too extensive to be deployed in large-scale epidemiological studies and therefore not often used.

Two highly viable methods for objective measurement of PA are the use of pedometers or accelerometers. These two methods have been used extensively in the field of PA research (39,48). Both measure PA from a mechanical point of view. Pedometers measure the number of steps taken by an individual, which is an easily interpretable metric. However, the intensity of the physical movement is not captured by a pedometer, but with an accelerometer (49). Accelerometers are now the most commonly used objective measure of PA (39), and are used in most large-scale epidemiological studies worldwide such as NHANES, UK biobank, and SCAPIS (50–52).

Accelerometers measure acceleration. Attached to the body, an accelerometer will capture the movement of the body segment. Usually, the accelerometer is worn attached to an elastic band around the waist and positioned over the right hip (39), since this is close to the body's center of mass (53). Wearing the accelerometer on the non-dominant wrist is also becoming common because of higher wear compliance but the accuracy of measuring PA is lower (39,54). The accelerometer can also be worn fixed to the mid-anterior thigh. The thigh placement is mainly used for classifying activity type rather than intensity e.g., sitting, standing, walking, bicycling (55), but can be used for intensity measurement as well (56). Other accelerometer positions used are shoe and ring positions (54,57). The accelerometer is typically worn for 7 days (51,52), which is considered to be sufficient to capture the normal variation in PA reasonably well (58).

Processing of accelerometer data

Modern accelerometers measure acceleration in three dimensions 30-100 times every second (39). In addition to movement, the acceleration caused by the gravity on Earth is also captured by the accelerometer. To get a useful metric of PA, this raw data has to be processed and reduced from the incredible detail that the accelerometer provides. The intensity and volume of PA are the most common

variables of interest (58) and are highly related to the way PA recommendations are communicated (29,59). This is often the average number of minutes spent at MVPA per day. To generate such a metric, the influence of gravity must be removed from the accelerometer data and the output must be relatable to energy expenditure.

The most widely used method of processing raw accelerometer data is to generate ActiGraph counts and calibrate the output toward energy expenditure measured by indirect calorimetry (58). The processing of ActiGraph counts briefly consists of filtering the raw acceleration to remove gravity and the potential influence of noise and aggregating the output to epochs of 1-60 seconds (60). There are numerous published calibrations between ActiGraph counts and energy expenditure, each with its specific cut-off for MVPA (58). Therefore, results from different studies investigating PA cannot be compared (61).

The ActiGraph counts method originates from an accelerometer designed in 1993 by the name “Computer Science and Applications Model 7164” (62). These sensors were later renamed to ActiGraph and models that are more modern have been released since, but still offer the same ActiGraph count output. Initially, only the vertical movement was considered whereas modern sensors measure the movement in all three dimensions, which is considered more accurate (63,64). The specifications of the processing of the ActiGraph counts have not been available until recently when Brønd et al. managed to replicate the process using an alternative accelerometer (60).

During light to moderate intensity PA, there is an approximately linear relationship between PA intensity and ActiGraph counts (65–68). This makes the ActiGraph counts a useful metric of PA within these ranges. At PA above moderate intensity on the other hand, equivalent to the walking-running transition, the relationship reaches a plateau (65,67,68). This obstructs differentiating PA equivalent to slow running from more intense PA. This is caused by the narrow passband of the frequency filter involved in the processing of ActiGraph counts whose purpose is to remove the gravity component and the potential influence of noise (67,68). The frequency filter has a high pass cut-off at 0.29 Hz and a low pass cut-off at 1.63 Hz, which means that only the part of the acceleration signal that is within this range would be included in the output (60). The step frequency during running is about 2.5-3.5 Hz and since this is outside the passband, a majority of the signal related to step frequency would be removed by the filter during running (69). Human locomotion generates acceleration up to approximately 10 Hz (67). Extending the low pass component of the frequency

filter to 4 or 10 Hz, the output continues to increase with higher PA intensity beyond slow running (67,68).

A disadvantage with a higher low pass cut-off could be the risk of capturing noise not related to PA, but the 4 or 10 Hz output has been shown not to capture more noise (68). However, the intensity classification between ActiGraph and the wider filters is highly different (68). The ActiGraph output is not able to discriminate between different levels of high-intensity PA (67). Although there is no association between ActiGraph output and PA intensity at this intensity level, calibrations usually assume an association similar to the association at low intensity. This implies that slow jogging and fast running will be mixed up to a substantial degree. The volume of PA generally decreases with increased intensity, which means that there will be more slow jogging mistakenly being classified as fast running compared to fast running mistakenly being classified as slow running. Overall, the consequence is that the ActiGraph output overestimates the volume of moderate, and especially vigorous, PA. (68). The 4 or 10 Hz processing method enables measuring the whole PA intensity range and provides a more accurate measurement of PA both from a mechanical and physiological point of view (67,70). However, this has not yet been validated in a free-living setting.

Statistical analyses

With improved methods of measuring PA, the whole intensity spectrum of PA is now possible to capture. Dividing the PA output in smaller intensity bins than the traditional light, moderate, vigorous, and very vigorous cut points, gives data of much more detail, and avoids the problem with multiple calibrations to MET values. Considering the entire PA spectrum will involve many variables that are highly multicollinear. This is a problem when investigating the effect of PA on health because traditional multiple linear regression cannot handle multicollinear independent variables. A proposed solution to this problem is to apply a partial least squares (PLS) regression that can deal with multicollinear variables. (71)

Background summary

The health benefits of PA are well-established, but there is less clarity regarding the health effects of specific PA intensities and the complex role of fitness in the association between PA and health. Accelerometers offer an objective way to measure PA, providing detailed data on individual activity levels. However,

traditional methods of analyzing this data underutilize the available information, making it difficult to gain a full understanding of the PA-health relationship.

Recently, there have been advances in data processing and statistical analyses that promise to better utilize accelerometer data. For example, extending the frequency filter range beyond the traditional 1.63 Hz low-pass cut-off provides a more accurate measure of PA across all intensity levels. Likewise, partial least squares regression can handle multicollinear variables, a common issue when considering the full PA intensity spectrum. These advancements offer the potential for a deeper exploration of PA intensity's importance and the role fitness plays in the association with health outcomes.

Aim

This doctoral thesis aimed to introduce and further develop multivariate statistical methods to analyze accelerometer-measured PA intensity. The thesis investigates how these analysis methods can utilize more of the detailed information from accelerometer data to provide novel insights regarding PA intensity and its relationship with cardiometabolic health, fitness, and role in intervention studies.

Research questions

1. What are the implications of more detailed analyses of accelerometer-measured PA intensity compared to traditional analysis methods? This is primarily investigated in Paper I and III.
2. What intensity of PA is most important in the association with health outcomes in various study designs when applying multivariate statistical methods? This is primarily investigated in Paper I, II, IV, and V.
3. Can multivariate statistical methods be used to study the role of PA intensity in the complex association between PA, fitness, and cardiometabolic health? This is primarily investigated in Paper IV and V.

Methods

This thesis introduces and develops multivariate statistical methods for analyzing accelerometer-measured PA intensity. Therefore, the methods section is an important part of the contribution of this thesis as it describes these methods in detail and their use in PA research.

Data collection

This thesis work does not include a collection of new data. Instead, already collected data was reanalyzed in collaboration with several different national and international research groups. The reanalyses included improved methods of PA data processing and statistical methods. Paper I analyzed data from the LIV 2013 study (72) and the SCAPIS pilot study (30,51). Paper II analyzed data from the I.Family study (73). Paper III analyzed data from the Bunkeflo study (74,75). Paper IV and V analyzed data from the Gothenburg site in the SCAPIS study (51).

LIV 2013

LIV is short for “Livsstil, prestation, hälsa”, or in English “Lifestyle, performance, health”. The LIV 2013 data collection started in 2013 and participants consist of a random sample of the Swedish population between age 20 and 65. The purpose of the LIV data collection is to investigate the effect of lifestyle on health. Participants have undergone several measurements of interest for this work including blood pressure, blood lipids, blood glucose, anthropometrics, fitness, and seven days of PA measurement with an accelerometer (not during sleep and water-based activities). The accelerometer used was ActiGraph GT3X worn over the right hip. The study design was cross-sectional and the total number of participants with accelerometer data was 921. (72)

SCAPIS

SCAPIS is short for “Swedish CARDioPulmonary BioImage Study”. A pilot study was performed in 2012 in Gothenburg to test the study design and was followed

up by a large-scale study including multiple sites all across Sweden between 2013 and 2018. The participants consist of a random sample of the Swedish population between ages 50 and 64. The purpose of the SCAPIS data collection was to investigate preclinical signs of pulmonary and cardiovascular disease to improve treatment and prevention. In addition to measurements of blood pressure, blood lipids, blood glucose, anthropometrics, and seven-day PA measurement with an accelerometer (not during sleep and water-based activities), the participants have also undergone measurements of lung function, electrocardiography, carotid arteries ultrasound, and MRI imaging, full body computed tomography imaging, coronary computed tomography angiography, among others. ActiGraph GT3X accelerometers were used for PA measurements and were worn over the right hip. The study design was cross-sectional, and the total number of participants was 30 000. In the Gothenburg subsample of approximately 6 000 participants, a sub-maximal test of fitness is also available. (51)

I. Family

The I. Family study is a multinational study of European children (73). The study extends the European IDEFICS study (76). In this thesis work a subsample of participants from Germany, Hungary, Italy, Poland, Spain, and Sweden was studied since these were the only sites that could provide raw accelerometry data. Cross-sectional data was collected during 2013-2014. In addition to the seven-day accelerometer measured PA, anthropometrics, blood pressure, blood lipids, and blood glucose was measured. ActiGraph GT3X accelerometers were used for PA measurements and were worn over the right hip for seven days (not during sleep and water-based activities). The number of individuals with valid PA measurements and at least one health-related outcome variable was 2592 and the mean age was 10.9 years (73).

Bunkeflo

The Bunkeflo study is sometimes also referred to as the Pediatric Osteoporosis Prevention Study (POP). The data collection took place in Malmö, Sweden, from 2001 to 2004. Participants were school children, and the first measurements were conducted in third or fourth grade where the mean age was 9.8 years. Follow-up measurements were conducted in the same children two years after the first measurements. PA was measured by the MTI model 7164 accelerometer

(Manufacturing Technology Inc.), which was worn on the right hip for four consecutive days.

The Bunkeflo study included an intervention to increase school time physical education in one school while children from other schools served as a control group with the standard amount of physical education. The intervention included 40 minutes of daily school-time physical education, totaling 200 minutes per week. The children in the control schools were scheduled for 60 minutes of school time physical education on one or two days. The activities during physical education in both groups followed the Swedish school curriculum and included a variety of running, jumping, ball games, etc. Accelerometer data collection was first implemented into the project 2 years after the start of the intervention with added PE. Therefore, the effect of the intervention cannot be investigated using accelerometer data. Instead, the study sample was used to demonstrate how improved statistical analysis of accelerometer data could find important information that traditional statistical methods miss. (74,75)

Measurements

Cardiorespiratory fitness

Cardiorespiratory or aerobic fitness is defined as maximal oxygen consumption during exercise. The gold standard for measuring fitness is an incremental exercise test where individuals cycle on a stationary bike or run on a treadmill until voluntary exhaustion. Simultaneously, oxygen consumption is measured from the difference in inspiratory and expiratory gas. This is rarely performed in studies on the general public since there are some risks associated with maximal efforts in untrained individuals and it requires advanced equipment. Therefore, a submaximal test is often used instead. The Ekblom-Bak test estimates maximal oxygen consumption by individuals cycling on a stationary bike at two predefined resistances while heart rate is being measured. A lower heart rate at higher resistance indicates a high oxygen uptake and fitness. (77)

Health variables

The main four risk factors of cardiometabolic disease are obesity, hypertension, insulin resistance, and dyslipidemia (11,12). In both adults and children, these risk factors tend to cluster and are in a combination called metabolic syndrome (12,15).

This thesis work mainly focuses on the prevalence of these risk factors in the general population as a sign of cardiometabolic health rather than the development of actual cardiometabolic disease.

Obesity is most often defined as a body mass index (BMI) above 30 kg/m², where BMI is calculated by dividing an individual's weight in kg by their height in meters squared. In children, age-standardized BMI is typically used since the body composition changes across childhood due to growth and maturity (78). Although obesity alone is considered a risk factor for cardiometabolic disease, excessive visceral adipose tissue is of particular risk (79). Therefore waist circumference, waist-to-hip ratio, waist-to-height ratio, or sagittal abdominal diameter are sometimes used as simple measures of visceral adipose tissue (80,81). Obesity can also be defined by body composition although no clear consensus on cut-offs is established. Obesity cut-offs that have been used however are body fat percentage above 25% and 35% to be defined in adult men and women respectively (82). In research, body composition is typically measured by bio-impedance or dual x-ray spectroscopy (DEXA), where the latter is much more accurate (83). DLW can also be used to estimate adipose tissue by measuring isotope dilution in the total body water (46).

Blood pressure can either be measured manually by the auscultatory technique or by semi- or fully automated devices based on the oscillometric technique. With both techniques, the blood pressure is measured with a cuff around the right upper arm over the brachial artery. The systolic blood pressure (SBP) refers to when the pressure is at its maximum and the diastolic blood pressure (DBP) refers to when the pressure is at its minimum in the blood vessels. Automated measurements are considered more accurate and usually, multiple measurements are performed to get an accurate reading. SBP is considered a more important risk factor for cardiovascular disease than DBP. (84)

Insulin resistance means that the body's ability to regulate blood glucose by insulin is impaired and could be considered a precursor of type 2 diabetes (12). Ideally, it is measured by a glucose tolerance test (12,85). However, it is more common to measure fasting levels of glucose and insulin from a blood sample (12). High levels of either glucose or insulin could indicate insulin resistance. These measures can also be combined with a homeostasis model assessment (HOMA) to estimate insulin resistance (85)

Dyslipidemia refers to an unfavorable composition of fatty acids and is also measured by blood samples. Specifically, dyslipidemia includes elevated levels of

triglycerides and low levels of high-density lipoprotein (HDL) compared to low-density lipoprotein (LDL) or total cholesterol. (12)

Since the combination of these risk factors also is of interest, they are often combined into scores indicating cardiometabolic health. Examples of this are z-score standardization or factor analysis (86,87). In addition, these scores are sometimes standardized by age and sex (16).

Accelerometer data

Raw data

An accelerometer is a sensor that measures acceleration, or more simply put, measures movement. Acceleration is usually expressed in *g* or *mg*, where one *g* or 1000 *mg* is equal to the gravity on Earth. In PA research, unprocessed acceleration is typically referred to as raw data.

The first accelerometer type used in PA research is based on a cantilever beam with a seismic mass at the end. When the sensor is moved, the seismic mass makes the cantilever beam bend, which produces an electric current that can be interpreted and stored on the sensor's onboard memory. This kind of accelerometer is called piezoelectric. Typically, acceleration is only measured in the vertical dimension with this accelerometer type. Since the properties of the cantilever beam change over time, the sensor has to be recalibrated regularly to ensure accurate capturing of acceleration. (88)

Modern accelerometers are instead based on capacitive technology (88). Capacitive accelerometers work by measuring the capacitance between two electrodes, where one is movable. When moved, the capacitance changes and produces changes in voltage that can be interpreted as acceleration and stored in the memory of the sensor. However, capacitive accelerometers also capture the acceleration of gravity. Although some variation in the calibration of these sensors is apparent, the response to acceleration is linear and robust to measurement error over time (89). Today, most of these accelerometers measure acceleration in three dimensions.

Accelerometers from ActiGraph have dominated the field of PA research (90). Initially by the piezoelectric accelerometer "Computer Science and Applications Model 7164" (CSA), and later by the capacitive accelerometers GT1M and GT3X. Only the GT3X is triaxial. The accelerometer signal is typically filtered to remove the influence of gravity and noise. In CSA, the filtering is hardware-based and the signal is filtered before being digitalized. In the capacitive sensors, on the other

hand, the signal is first digitalized and then filtered using a digital bandpass filter (88). Although the output is claimed to be comparable between these sensors, there are differences apparent (91,92).

The details of the processing of raw accelerometry data made by ActiGraph are proprietary. However, with the modern GT3X sensors, raw accelerometer data is stored on the device and is being processed, including filtering, in ActiGraphs computer software ActiLife. This makes it possible to use the raw data instead of the processed data. The raw data is compressed in a specific format in “.gt3x” binary files. These files can either be exported through the ActiLife software or be unpacked by other software using available file specifications (93). This has been utilized by Brønd et al. to replicate the ActiGraph processing by inputting artificial accelerometer signals into ActiLife (60).

In addition to the limitations of the ActiGraph processing raised in the background of this thesis, there is an increasing interest in transparent, open-source, methods of PA measurement (94). Axivity AX3 (Axivity Ltd., Newcastle upon Tyne, UK) is an open-source accelerometer that is being increasingly used in PA research, e.g. in the UK biobank study with more than 100,000 participants (52). Axivity AX3 is a relatively cheap, small (23 x 32.5 x 7.6 mm, 11 g), waterproof, triaxial, capacitive accelerometer that comes with open-source software for data initialization and extraction. Furthermore, there is also available open-source software for processing raw accelerometry data. The GGIR package for R includes software for processing accelerometer data from several different sensors including GT3X and AX3 (95).

The size of a raw accelerometer data file from the ActiGraph GT3x device with .gt3x format from one week of measurement is typically around 40 MB. This file format is highly compressed to save storage space and each sample from a single axis is stored as a 12-bit value (93). For comparison, when working with the data in computing software like Matlab or R, this data is typically stored in the computer's random-access memory with what is referred to as “double precision” which uses 64 bits. Furthermore, the GT3x accelerometer can enter “idle sleep mode” when no movement occurs for a few minutes. The idle sleep mode implies that the accelerometer pauses the writing of new samples to the internal memory until the accelerometer moves sufficiently to overcome a certain cut-off. This also helps to reduce the size of the .gt3x files substantially. Still, the accelerometer files from the SCAPIS study with approximately 30,000 participants require more than 1 TB of storage.

Data processing

ActiGraph accelerometers use a sampling rate of 30 Hz as standard. Although GT3X accelerometers could be initialized to record movement at a different sampling rate, it is recommended to use 30 Hz or a multiple of 30 Hz (60 or 90 Hz) since the results could differ otherwise (96). AX3 offers a wide range of sampling frequencies, from 12.5 to 3200 Hz, but not 30 Hz specifically. However, the raw data can be resampled to 30 Hz upon data extraction in the accompanying software.

The processing of ActiGraph counts involves the following steps performed on 30 Hz raw data from each axis separately (60):

1. A 15 Hz aliasing filter to make sure that the signal is compatible with the Nyquist-Shannon sampling theorem.
2. The ActiGraph-specific band-pass filter with high-pass and low-pass half-power cut-offs of 0.29 and 1.63 Hz respectively. This removes the influence of gravity as well as most of the signal related to PA of vigorous intensity.
3. Downsample to 10 Hz.
4. Truncate to ± 2.13 g. Higher values than this are set to 2.13 g.
5. Absolute value. Negative values are turned to positive.
6. Dead band at 0.068 g. Lower acceleration than this is set to zero.
7. Converting output in g to 8-bit resolution by dividing by 0.01664. Since the range of the sensor is ± 2.13 g, this means that $2 \times 2.13 \text{ g} = 4.26 \text{ g}$ must be stored with 8-bit resolution. This resolution is equivalent to a range of $2^8 = 256$, which implies that the resolution is $4.26 \text{ g} / 256 = 0.01664 \text{ g} = 16.64 \text{ mg}$. This is how the unit ActiGraph counts is defined.
8. Aggregation of 1-60 s epochs. Samples are summed up with a specific resolution which means that the output is dependent on epoch length.

Some of the steps of the ActiGraph processing might seem counterintuitive from a measurement perspective but are likely performed to ensure backward compatibility from the GT3X to the CSA sensor. The improved frequency extended method (FEM) primarily changes the low-pass half-power cut-off from 1.63 Hz to 4 or 10 Hz. However, some additional changes to the processing are also made. Raw 30 Hz data from each axis is processed in the following steps (68):

1. Bandpass filter with high-pass and low-pass half-power cut-offs of 0.29 and 4 or 10 Hz respectively. This removes gravity and enables the capturing of all the information related to PA.
2. Truncate to 6 *g*. This is only done with AX3 accelerometers, which have a range of ± 8 *g*, to make the results comparable with GT3X accelerometers which have a range of ± 6 *g*.
3. Absolute value. Negative values are turned to positive.
4. Dead band at 0.068 *g*. Lower values than this are set to zero.
5. Multiplication by 1000 to express the output as *mg*.
6. Mean acceleration of each 1-60 s epoch. This means that the output is not dependent on epoch length.

Data from each axis is often combined to a vector magnitude after processing. In all the analyses in this thesis, the triaxial vector magnitude has been used. The high-pass half-power cut-off is kept the same in both methods. This cut-off removes information in the signal that is repeated less often than once every $1 / 0.29 = 3.4$ seconds. Since the gravitation is constant, it is removed by this part of the filter. This cut-off also suggests that activities with a cycle of up to 3 seconds are captured. A shorter epoch length than 3 seconds would therefore capture different parts of the same activity and lead to more noise in the output. However, short epoch lengths capture more intermittent and sporadic PA and have been suggested to be better to use when studying children (97,98). An epoch length of 3 seconds has been used throughout this thesis to balance the ability to capture sporadic PA with the risk of capturing noise.

Apart from the ActiGraph method of processing raw accelerometer data, another common method is Euclidian Norm Minus One (ENMO). ENMO is the main processing method used in the open-source R package GGIR. This method consists of calculating the Euclidian norm (vector norm) of the accelerometer signal from all three axes, and then subtracting 1*g* to remove the influence of gravity (99). No frequency filter is involved in the processing which could make the accelerometer output more sensitive to capturing noise. In addition, this method is sensitive to deviations in the calibration of the raw accelerometer data.

However, this can be solved by applying an autocalibration algorithm to the raw data (89).

Processing accelerometer data requires substantial computational resources. Even with a high-performance personal computer, each accelerometer file takes several minutes to process. The computer code used for analyses can be optimized by vectorization and adapted to be run on several computer cores in parallel to speed up processing. Still, processing the entire SCAPIS material with 30,000 accelerometer files takes more than a week of constant processing by several computer cores. High-performance computing clusters can be used to speed up this process further, like the Swedish National Infrastructure for Computing SNIC (100).

Calibration

The processed accelerometer output needs to be calibrated to make it interpretable. This has typically been done by studying the relationship between accelerometer output and energy expenditure in METs while individuals walk and run on a treadmill. Accelerometer output cut points representing 1.5, 3, 6, and sometimes 9 METs, equivalent to light, moderate, vigorous, and very vigorous PA, are found by fitting a linear regression to this relationship. The most widely used cut-points are developed by Freedson et al. (101), and Evenson et al. (98), for adult and children populations respectively.

There are numerous other approaches to calibration between accelerometer output and METs. Curvilinear regression has been used based on higher-order regression models or smoothing splines (58). With improved methods of accelerometer data processing, it is apparent the association between accelerometer output and energy expenditure is non-linear, because of the walking-running transition (67,68). Other methods include the receiver operating characteristic curve (ROC) which maximizes the sensitivity and specificity of the calibration (58). More advanced calibration models including machine learning techniques have also been developed (102). These models seem to improve measurement by wrist-worn accelerometers substantially, but no consistent improvement with hip-worn accelerometers has been shown (102).

Accelerometer output is not calibrated directly to absolute energy expenditure since absolute energy expenditure is highly different between individuals, mainly because of body size. METs is a measure relative to the individual resting metabolic rate and are more comparable between individuals' performing activities at the same intensity. However, from a physiological and biomechanical

perspective, energy expenditure is not expected to increase in proportion to resting metabolic rate during increased intensity PA but rather in proportion to body weight. Therefore, it has been suggested to calibrate the accelerometer output to VO₂net instead. VO₂net is defined as oxygen consumption per kg body weight minus the oxygen consumption standing or at rest. In children, VO₂net would be more comparable to adult PA intensity since children have a relatively large resting metabolic rate. (36,70)

The main problem with calibration, however, is not necessarily the accuracy of calibration models, but rather that the results differ substantially depending on what cut points are used (61). The cut-points are also a very crude measure of PA, that misses a lot of the detail in the PA data (71,103). Increasing the resolution of PA intensity from 4-6 cut-points to a 20+ bin intensity spectrum overcomes both these problems by presenting the results in much more detail, not dependent on specific cut-points. Typically, the resolution of the spectrum is around 0.5 METs, which refers to how far apart the bin edges that define the variables in the intensity spectrum are. However, a higher resolution does lead to new problems with multicollinearity between the variables representing the PA spectrum, but multivariate statistical methods and PLS in particular have been shown to handle this problem well (71).

Valid days

The processing of PA data further includes defining what is a valid measurement. This is done by first detecting periods of non-wear followed by comparing this to a criterion of the number of hours of wear time for a valid day and finally a criterion of the number of valid days for a valid measurement. There are multiple methods for detecting non-wear time (58). The most common is to define non-wear time as 60 consecutive minutes of zero output with an allowance of up to 2 minutes of activity of less intensity than the sedentary cut-point (104). Furthermore, different criteria of valid day and valid measurement have been used, but at least 10 hours per day for at least 4 days are often used (58). These methods and criteria for non-wear, valid day, and valid measurement have been used throughout the analyses in this thesis. There is a larger day-to-day variation in high-intensity PA compared to low-intensity PA, which means more days of measurement are required to reliably capture moderate, and especially vigorous, intensity PA (105).

Statistical analyses

Analyzing physical activity intensity

Measurement of PA by accelerometers results in variables representing time spent at different PA intensities. These variables are used in statistical models for analyzing associations with health-related outcomes or to compare PA between groups. Since most health benefits are assumed to be related to time spent at MVPA, the simplest statistical analyses of PA intensity are to investigate the association between MVPA and health outcomes by bivariate correlation or regression analysis or to compare MVPA between groups using t-tests. More variables representing different PA intensities, including sedentary time, can be used in statistical models. However, this might result in too much collinearity between the independent variables (106).

Multicollinearity

Multicollinearity between the variables representing time spent at different PA intensities has two different causes. The first reason for multicollinearity is what is referred to as the closure problem; all research participants are limited to 24 hours per day (107,108). If an individual spends more time at a specific PA intensity, the time spent at other intensities must decrease. For this reason, time spent sedentary is inversely collinear with time spent at light intensity (109). Two methods that are specifically aimed at handling the closure problem are isotemporal substitution (108), and composite data analysis (107,110). However, these methods do not handle all potential sources of collinearity (106).

The second reason for multicollinearity is more of a behavioral aspect. An individual who performs much vigorous intensity PA is more likely to perform more very vigorous activity as well. This is even more prominent when PA intensity is studied with higher detail by the intensity spectrum since neighboring intensity variables essentially capture the same activity (111). Collinearity above 0.6 is sometimes considered problematic in multiple linear regression, whereas others suggest that collinearity is only a problem above 0.9 (108). However, in a high-resolution intensity spectrum, 24% and 7% of the correlation coefficients between the variables could be above 0.7 and 0.9 respectively (111).

The main problem with collinearity comes from the multiple regression model being unstable when the independent variables are collinear. Essentially, multiple linear regression fits a plane, or hyperplane depending on the number of independent variables, to the data to explain the variation in the dependent variable. This is visualized in Figure 1 with variables representing age and time spent at MVPA on the horizontal axes and fitness on the vertical axis. The data points are scattered with a variation over the two horizontal axes independent of each other and there is sufficient variation to fit a plane to this data.

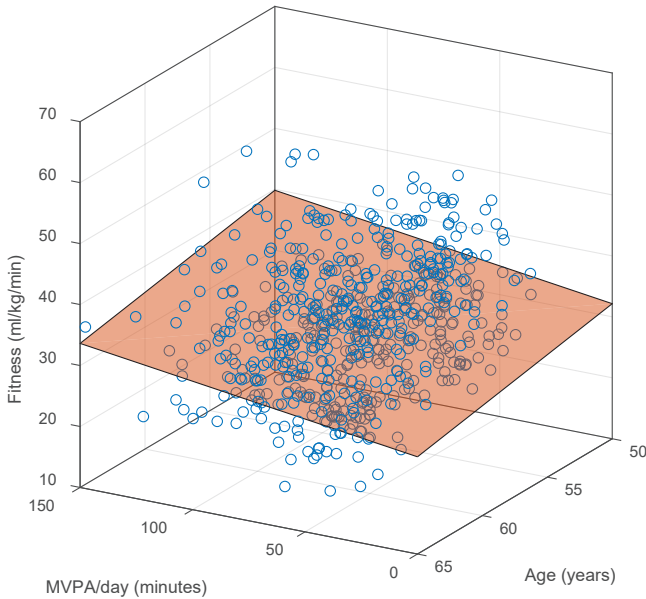


Figure 1. Multiple regression.

Visualization of a multiple regression with MVPA and age as independent variables on the horizontal axes and fitness as dependent variable on the vertical axis. Data from the LIV and SCAPIS pilot study.

If the independent variables are collinear, the variation follows a straight line instead of being spread out over a plane. When trying to fit a plane to the data points that are following a straight line, the plane becomes unstable and is highly affected by multivariate outlier data points that fall outside of the straight line that the rest of the data follow. This instability might cause the plane to tilt heavily to either side of the straight line and the interpretation of the association of the two independent variables changes drastically. This is referred to as the regression coefficients being inflated (112). A visualization of a multiple linear regression between two collinear PA variables and fitness is shown in Figure 2.

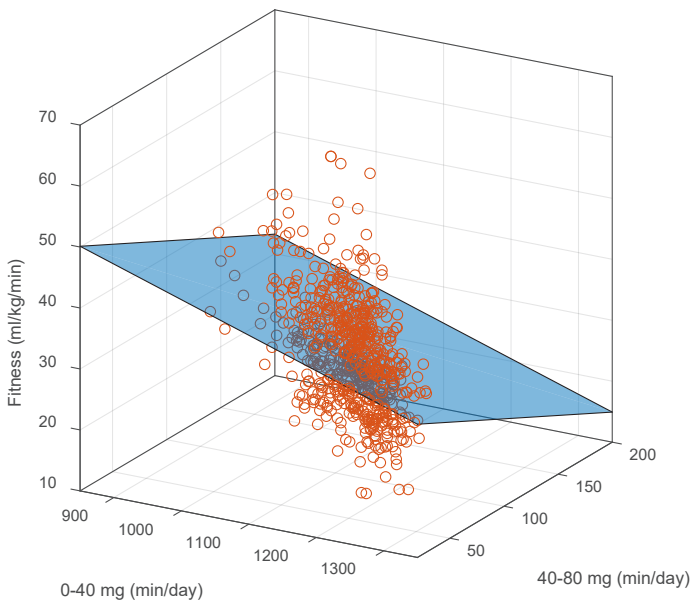


Figure 2. Collinear multiple regression.

Visualization of a multiple regression with two collinear PA variables on the horizontal axes and fitness as dependent variable on the vertical axis. Data from the LIV and SCAPIS pilot study.

The PLS method, simply put, fits a line to the data instead of a plane. The line is found in a way that maximizes the covariance with the response variable, fitness in this example. This is visualized in Figure 3. Typically, around 20 different PA variables are used in a PLS model, and one line is fitted to all these variables. Each latent variable (PLS component) is represented by a different line.

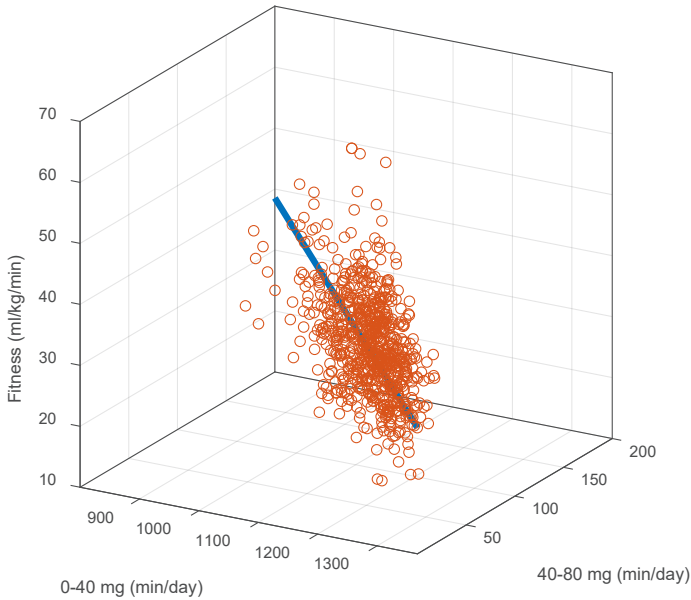


Figure 3. PLS regression.

PLS regression visualized as a line fitted to two collinear PA variables on the horizontal axes and fitness as dependent variable on the vertical axis. Data from the LIV and SCAPIS pilot study.

An attempt to, somewhat simplified, describe PLS in relation to other methods of analyzing associations between multiple variables is shown in Figure 4 and described below. PA1 and PA2 refer to two variables representing time spent at different intensity levels.

- Bivariate correlation between Health and PA1 is represented by A+B and the correlation between Health and PA2 is represented by B+C.
- Multiple linear regression with the PA variables as independent variables and Health as dependent variables results in an R^2 value that represents A+B+C. The regression coefficient for PA1 would be A and a part of B and the regression coefficient for PA 2 would be C and the remaining part of B.
- Partial or semi-partial correlation between PA1 and Health represents A. The difference between partial and semi-partial is whether this is put relative to the overall variation in Health (semi-partial) or just the part of the variation in Health that does not overlap with PA2 (partial).
- PLS essentially represents B. However, B is found in a way that maximizes the covariance in Health. Furthermore, the use of multiple latent variables (PLS components) allows for finding an initial B, then removing this variation from the variables and trying to find additional overlap for an additional latent variable.

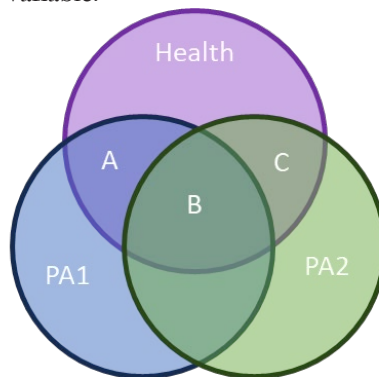


Figure 4. Illustration of covariance.

Illustration of covariance between physical activity (PA) and health variables. Circles represent the variation in the variables and the overlap between the circles represents the covariation. Health represents a health-related variable and PA1 and PA2 represent two PA intensity variables.

Partial least squares regression

Partial least squares regression (PLS) decomposes the predictor variables into one or more latent variables, similar to principal component analysis. However, principal component analysis maximizes the explained variance in the original predictor variables on each latent variable, whereas PLS maximizes the latent variables' covariance with the response variable and is, therefore, more suitable for use in regression (113). Selecting the number of latent variables used should be done by cross-validation (113), where Monte Carlo resampling with 1,000 repetitions has been suggested to be an effective and transparent method (114). A cut-off can be set to ensure that the number of components used is significantly better than a model with fewer components.

Like in traditional multiple linear regression, the overall predictive strength of the PLS model is typically expressed as the explained variance in the outcome variable (R^2). However, in multiple linear regression, the regression coefficients can be interpreted to determine the predictive influence of the different predictor variables. Although regression coefficients can be obtained from the PLS method, these coefficients do not essentially represent the predictive ability of the original variables. PLS regression coefficients are sensitive to covariation between the predictive variables that are not necessarily related to the response variable (115). To facilitate interpretation, a selectivity ratio is calculated for the predictor variables. The selectivity ratio represents the explained variance in the predictor variables from the latent variables. It is calculated by combining the predictive variation of the latent variables to a single predictive component by target projection. Subsequently, the explained variation in the predictor variables from the target projected component is divided by the overall variation in the predictor variables (115). Furthermore, the selectivity ratio can be multiplied by the explained variation in the response variable to obtain the (multivariate) explained variation in the response variable from each predictor variable (106). As an alternative, the square root of the explained variation from each predictor variable can be presented, which represents multivariate correlation coefficients (106). The confidence interval of the selectivity ratio and explained variance in the response variable can be obtained by bootstrapping with 1,000 repetitions. The PLS model is illustrated in Figure 5.

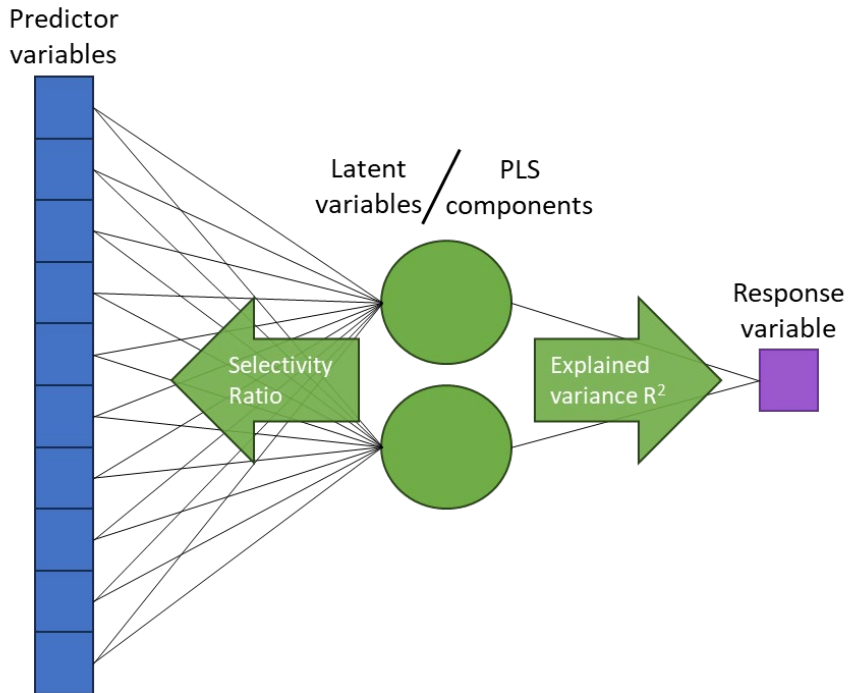


Figure 5. Illustration of the PLS model.

The selectivity ratio represents the explained variance in the predictor variables by the latent variables and the explained variance (R^2) refers to the explained variance in the response variable by the latent variables.

The predictor and response variables are standardized to a mean of zero and a standard deviation of one before they are used in the PLS model. Therefore, the selectivity ratio should be interpreted as to what extent the variation in the predictor variables explains the variance in the response variables and not the influence of e.g., one minute of activity at a specific intensity. However, the selectivity ratio can be unstandardized by division with the standard deviation of the original variables to obtain an estimate of the effect of a one-minute change in PA. In addition, the explained variance of the response variable is shared between the predictor variables. Hence, this explained variance cannot be interpreted as independent from the other predictor variables. Instead, the influence of all the predictor variables should be interpreted as an overall pattern related to the response variable. (106)

Since a PLS model finds latent variables that maximize the covariance with the response variable it will most likely be able to explain some degree of variance in

the response variable by chance. Therefore, a traditional test of the statistical uncertainty (p-value), where the null hypothesis would be that the model is not able to explain any variance in the response variable, cannot be used. Instead, permutation tests can be applied to determine the statistical uncertainty of the PLS model (116). In permutation tests, the predictor and response variables are scrambled between individuals and then used for developing additional PLS models. If there is a meaningful association between the predictor and response variables, the initial model should be able to explain significantly more variation in the response variable compared to the permuted/scrambled models (116). To establish a statistical uncertainty (p-value) less than 0.01, 10,000 permutations are required (117).

The use of PLS regression can be extended for use with a dichotomous response variable and is then called PLS discriminant analysis (PLS-DA). The PLS model will find latent variables that maximally discriminate the response variable. In this case, the correct number of classifications should be used for assessing the predictive performance of the model instead of explaining variance in the response variable. For analysis of PA patterns of independent measurements, the response variable can be coded as -1 and 1. PLS-DA can also be applied for analyzing paired measurements by using the difference in the original variables as predictor variables. (118)

For the analysis of the association between PA and health, PLS can be applied with the PA intensity spectrum as predictor variables and a health variable as the response variable. It could be suitable to divide the PA intensity spectrum into 15-35 segments depending on the size of the dataset (64,119). The response variable could either be a single health variable or many health variables combined to a standardized score (86,119). The result from the PLS regression would be the overall explained variation of the model (R^2) and the association of each segment in the PA intensity spectrum to the health variable displayed as a selectivity plot, see Figure 6 for an example. A p-value representing the statistical uncertainty of the PLS model can be obtained from a permutation test (116). PLS can also be used for comparing PA patterns between groups using PLS-DA. Both independent groups and paired measurements can be investigated (118).

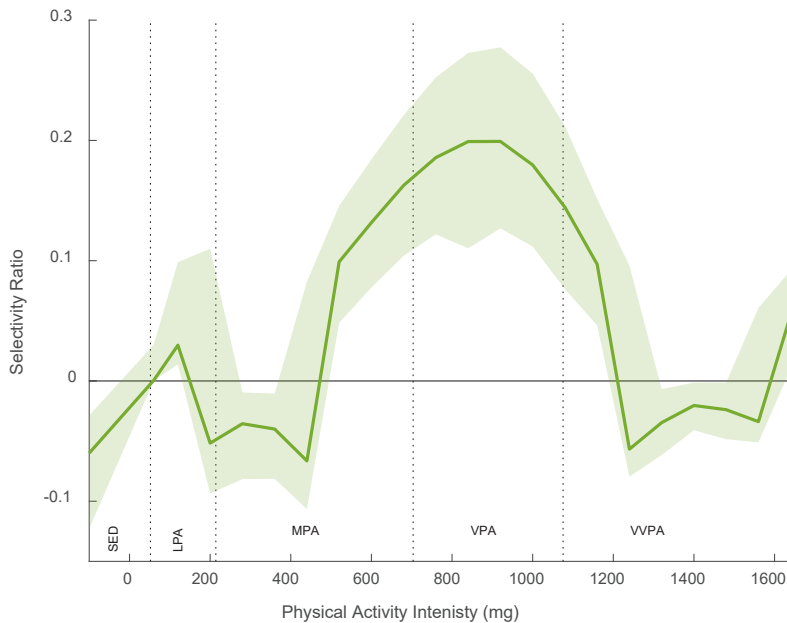


Figure 6. Example of a selectivity ratio plot.

Example of a selectivity ratio plot of the relationship between the physical activity (PA) intensity spectrum divided into 22 segments and a composite score of cardiometabolic health variables. The acceleration related to PA intensity is displayed on the x-axis and the selectivity ratio is displayed on the y-axis. The selectivity ratio represents to what degree each intensity level contributes to the total explained variance ($R^2 = 18.3\%$). A negative selectivity ratio indicates that more time spent at the PA intensity is associated with a lower composite score (unhealthy) and a positive selectivity ratio indicates that more time spent at the PA intensity is associated with a higher composite score (healthy). Shaded areas represent 95% confidence intervals. Figure is based on data from Paper II.

In PA research, PLS has mainly been applied in epidemiological studies (97,111,120–122). In this kind of study, the associations between PA and health outcomes are typically affected by numerous different confounding variables that the researchers would like to control for. In multiple linear regression, this is done by entering the confounding variables as additional independent variables. This is a convenient and relatively easy-to-interpret approach since the regression model divides the explained variation between the independent variables. In PLS analysis, on the other hand, the predictor variables are not independent of each other. Confounding variables can be entered as additional predictor variables in the PLS

model, but must then be interpreted together with the PA variables which is difficult (111,122). A more common approach is to remove the variation in the response variable related to the confounding variable before entering the response variable into the PLS model (119–121). This is done by a multiple linear regression with the confounding variables as independent variables and the response variable as dependent variable and the residuals from the regression model are used as response variables in the PLS model. The downside of this approach, however, is that all the explained variation in the response variable that is shared by the confounding and PA variables are ascribed to the confounding variables. A solution to the problem of considering confounding variables in PLS analysis is to use PLS structural equation modeling (PLS-SEM) to build more complex models (123). In addition, PLS-SEM can investigate more intricate relationships between variables including mediation.

Structural equation modeling

Partial least squares structural equation modeling (PLS-SEM) is a common tool in social sciences for examining intricate relationships among various variables. It merges latent variable calculation from PLS with path modeling to construct sophisticated models (123). PLS-SEM is typically employed for contrasting different theoretical models based on their data fit and for investigating indirectly measured unobservable constructs. When applied to PA intensity analyses, PLS-SEM provides a straightforward approach to manage the collinearity of PA variables by treating them as latent constructs, while building more complex models. The emphasis of these analyses isn't on the model-data fit, as with many PLS-SEM applications, but on the interrelationships of the variables, similar to traditional multiple regression. In studying the link between a group of PA intensity variables and a single health outcome variable, PLS-SEM and PLS regression yield essentially identical results. However, PLS-SEM enables a more intuitive understanding of covariates in the PA-health variable relationship.

Creating PLS-SEM models requires specifying a measurement model and a structural model. The measurement model identifies what constructs the measured variables represent, and the structural model outlines these constructs' relationships. To use single variables in a manner similar to multiple regression, such as control variables like age and sex or a health-related outcome, they're defined as single-item composites in the measurement model. For a multivariate analysis of PA intensity, the variables are first defined as one multi-item composite,

with the variables representing the PA intensity spectrum. To allow multiple latent variables representing PA (PLS components), additional multi-item composites can be created. The variables for these further composites are derived by deflating the previous PA variables (113). Deflation involves predicting the PA variables from the scores and loadings of the previous composite and then subtracting the predicted values from the original ones. This process eliminates variance related to the previous composite, permitting the deflated PA variables to define an additional composite. Subsequently, a higher-order composite is established based on the multiple PA composites. The number of PA composites (PLS components) is chosen via Monte Carlo resampling, in the same way used in simple PLS analysis (114). Like in PLS analysis, the influence of different PA intensities can be presented as a selectivity ratio (115). Figure 7 illustrates a PLS-SEM model of the association between PA and health with potential mediation through fitness while also controlling fitness and health for age and sex.

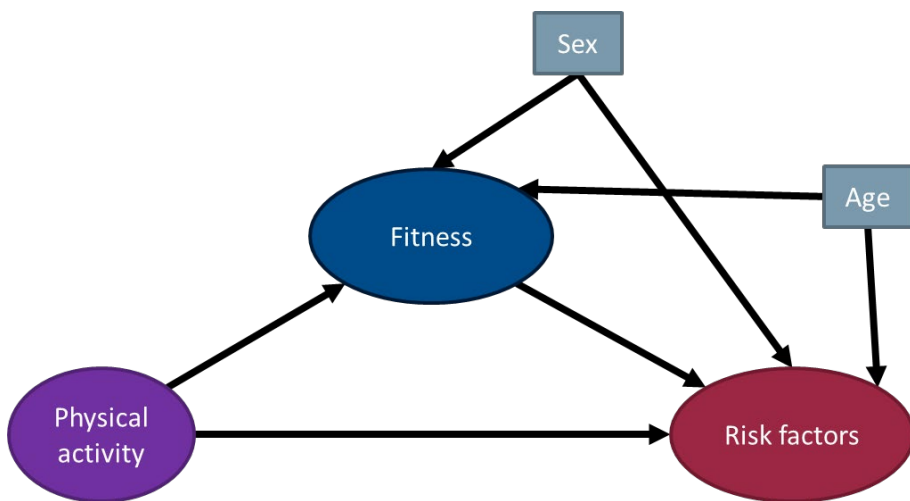


Figure 7. Illustration of a PLS-SEM model.

PLS-SEM model of the association between PA and health with potential mediation through fitness while also controlling fitness and health for age and sex.

Moderation and mediation

Previous research suggests fitness has both a moderating and mediating role in the association between PA and health. Moderation refers to the effect of PA on health being different depending on fitness level (2). An example of this is that less intense PA intensity is thought to be sufficient to improve health in low-fit

individuals compared to high-fit individuals who require PA of higher intensity. Mediation on the other hand refers to the causal pathways of the effect of PA on health. In PA research, it is debated to what degree the health benefits of PA are due to better fitness or if the effect of PA on health is independent of fitness level (2). Previous studies have suggested other mechanisms where the benefits of PA are mediated through other variables than fitness, including behavioral aspects (124).

Software

Matlab

Matlab (MATrix LABORatory; MathWorks, Natick, MA, USA) is a high-level programming environment and numerical computing software widely used in scientific and engineering disciplines. It provides a powerful set of tools for data analysis, visualization, algorithm development, and numerical computations. Matlab is especially efficient in handling matrices and arrays, which is particularly suitable for processing accelerometer data and multivariate statistical analyses. To fully utilize this efficiency, the code should be vectorized and loop-based scalar operations should be avoided (125). Matlab offers an extensive library of prebuilt functions and toolboxes, enabling quick implementation and analysis of various problems as well as extensive visualization capabilities.

The toolboxes mainly used in this thesis are the Signal Processing Toolbox and the Statistics and Machine Learning Toolbox. Specifically, the frequency filtering of the accelerometer data has been implemented as digital filters (126), and the PLS regression models have been implemented using the function `plsregress` (127). The Matlab versions used in the thesis work range from R2018b to R2022b.

R

R (R Core Team) is an open-source statistical programming language and software environment widely used in scientific research and data analysis. Like Matlab, R is suitable for analyzing large amounts of data in vector or matrix form. Although the basic functionality of the software is not as extensive as Matlab, R benefits from a large and active user community, which contributes to its vast collection of packages and provides support through forums and online resources. This collaborative ecosystem ensures that R remains up to date with the latest statistical

techniques and data analysis methods. In this thesis work, R has only been used to develop more complex statistical models in the form of PLS-SEM models. However, the most widely used open-source software for analyzing accelerometer data, GGIR (95), is based on R. The R version used was v4.1.2.

SEMinR

The PLS-SEM models were created in R using the *semplr* package (v2.3.2; Ray S, Danks N, Valdez A 2022)(128). It is an open-source software capable of analyses using several different structural equation modeling techniques in addition to PLS. The software is accompanied by a freely available e-book with extensive information on PLS-SEM in general and how to use software specifically (123).

Paper-specific methods

Paper I

Data collected in LIV 2013 and SCAPIS pilot were analyzed. Complete data on PA and cardiometabolic health were available from 725 participants. Accelerometer data collected from the hip was processed to 10 Hz and ActiGraph output. Similar to a study in children by Aadland et al. 2018 (119), six variables of cardiometabolic health were combined to a composite score (CS), and the PA was divided into an intensity spectrum of 22 bins. The variables being analyzed were SBP, blood triglycerides, total cholesterol to HDL ratio, insulin resistance from HOMA, waist-to-height ratio, and fitness.

The CS was calculated by standardizing each variable to the within-sample standard deviation and mean and taking the mean of these variables for each participant (86). All variables except fitness were reversed (by multiplying with -1) so that a positive CS indicated good cardiometabolic health. The relationship between the PA intensity spectrum variables and the cardiometabolic health CS was analyzed using PLS regression and presented as a selectivity ratio plot where the association between each PA intensity bin and the CS was shown (115).

The hypotheses were 1) that the overall explained variation in CS would be higher with the 10 Hz output compared to the ActiGraph output, and 2) that the strongest association between PA and CS would be shifted to vigorous intensity PA with the 10 Hz output compared to the ActiGraph output.

Paper II

Data from the I.Family study was analyzed. Accelerometer data from the hip was processed to 10 Hz output and divided into an intensity spectrum. Measures of cardiometabolic disease risk factors used in the analyses in the paper were BMI, insulin resistance from HOMA, HDL, SBP, and DBP. In addition, age and sex-standardized measures of BMI, waist circumference, SBP, DBP, blood glucose, and triglycerides were used to quantify metabolic health as a CS.

The standardized PA patterns were compared between the different countries included in the paper and compared between children in different BMI status groups. Furthermore, the association between the PA intensity spectrum and the different cardiometabolic disease risk factors and CS was investigated by PLS regression. Because of the diversity in the data coming from different countries, confounders were added to the analyses. Age, sex, country and parents' education, and socio-economic status were considered confounders. The addition of confounders into the PLS regression model was a novel component compared to Paper I but introduced challenges to the statistical analyses and the interpretation. The confounders were therefore treated in two different ways. First, three different PLS models were compared, one with only PA as independent variables, one with PA and confounders as independent variables, and one with only confounders as independent variables. Second, the confounders were used as independent variables in a traditional multiple linear regression with health variables as dependent variables. The residuals from this regression were used as dependent variables in a PLS regression with PA as independent variables. This solution of handling confounders in PLS regression is not optimal and an alternative method was targeted in Paper V.

The Swedish National Infrastructure for Computing (SNIC) at Chalmers Centre for Computational Science and Engineering (C3SE) was used for processing the accelerometer files in a high-performance computing cluster. Because of restrictions on the use of personal data related to cardiometabolic health, this data was not allowed to be moved outside of Germany. Therefore, a virtual private network (VPN) was used to access a remote computer stationed in Germany to perform the statistical analyses remotely.

This paper hypothesized that the methods of data processing and statistical analyses would provide detailed information about the association between PA and cardiometabolic disease in children.

Paper III

Data from the Bunkeflo study was analyzed. Measured PA was compared between children from the intervention school (intervention group) and the control schools (control group) as an independent group analysis. In addition, PA was compared between the first and second measurements, separated by two years, among the same individuals in the intervention group as a paired analysis. Previous studies using traditional cut-points and statistical methods have not found any significant difference in PA levels between the intervention and control groups. In this paper, the results from traditional and multivariate statistical methods were compared.

PA was measured by the MTI model 7164 accelerometer (Manufacturing Technology Inc.), also known as ActiGraph model 7164. This is a uniaxial accelerometer solely measuring movement on the vertical axis. In addition, this accelerometer does not provide raw accelerometer data and the analyses were therefore limited to using accelerometer data processed by the ActiGraph filter. This processed accelerometer output had an epoch length of 10 seconds. Traditional cut-points (98), as well as an intensity spectrum consisting of 30 bins, were used to generate PA intensity variables.

Traditional analyses were conducted using the PA variables generated from the cut-points and independent and paired samples t-tests for the independent and paired group comparisons respectively. PLS-DA was used for the multivariate analyses to compare the independent intervention and control groups as well as the two paired measurements of the intervention group two years apart.

This paper hypothesized that using PLS-DA to analyze the PA intensity spectrum would be able to identify previously undetected differences in PA between the intervention and control groups as well as PA differences between the two measurements of the intervention group.

Paper IV

Data collected in the Gothenburg sub-sample of SCAPIS was analyzed, where 4,234 individuals provided valid data for all variables investigated. The reason for only including this subsample was that it includes measurements of fitness as opposed to the entire SCAPIS study. Fitness was estimated from a submaximal Ekblom-Bak bike ergometer test (77), and the sample was divided into tertiles based on fitness. Accelerometer data from the hip was processed to 10 Hz output and divided into two different intensity spectra. One spectrum was based on an absolute measure of PA (METs) and the other spectrum was based on a relative

measure of PA (proportion of oxygen consumption relative to maximal oxygen consumption). A CS was calculated by combining sex-standardized measures of waist circumference, HDL, triglycerides, HbA1c, and SBP.

The associations between the absolute and relative PA intensity spectra and fitness as well as CS were calculated by PLS regression for each of the fitness groups separately. Selectivity ratio plots were displayed for each fitness group indicating what intensity of PA was strongest associated with cardiometabolic health. This analysis can be considered an investigation of the moderation of the association between PA and health by fitness. In addition, the total amount of MVPA and fulfillment of the recommendations on 150 minutes of MVPA per week were calculated based on absolute and relative intensity.

The hypothesis was that the association between PA and CS would be shifted towards lower intensity PA in individuals with low fitness and towards higher intensity PA in individuals with high fitness when considering absolute intensity. In addition, it was hypothesized that the associations would be similar when considering relative intensity.

Paper V

The same sample from the SCAPIS with 4,234 individuals used in Paper IV was analyzed. Fitness and health-related outcome variables were measured in the same way as in Paper IV, including CS calculation. Recent results suggest a 4 Hz filter would be better than a 10 Hz filter for capturing health-related PA, however, both are better than the ActiGraph filter (129). Therefore, a band-pass filter with a 4 Hz half-power cut-off was used for processing the accelerometer data. PA pattern was represented by an intensity spectrum consisting of 22 variables up to approximately 10 METs.

PLS-SEM was used to develop six different models for two purposes. First, to demonstrate how PLS-SEM can be used for incorporating and interpreting confounding variables in multivariate analyses of PA intensity. Second, to investigate the mediating role of fitness in the association between PA and health. The models were:

1. Simple PLS model of the association between PA and CS.
2. Model of the association between PA and CS while controlling for sex and age.
3. Model of the association between PA and fitness without controlling for sex and age.

4. Model of the association between PA and fitness with controlling for sex and age.
5. Model of the association between fitness and CS while controlling for age and sex.
6. Final model of the association between PA and CS with mediation through fitness while controlling fitness and CS for sex and age.

The hypothesis was that a significant part of the association between PA and CS would be mediated through fitness and that the PA pattern from the PLS model would be different depending on whether fitness was considered in the model or not.

Ethical considerations

Research can only be performed if the benefits outweigh the risks, and if the results cannot be achieved with alternative methods that are associated with a lower risk (130). This includes risks to the participant's health, safety, and personal integrity. Measurement of PA by an accelerometer is practically risk-free from a physical perspective but it could involve personal integrity risks. Current methods for analyzing and aggregating raw accelerometry data do not pose a high risk for personal integrity since only the average time spent on different PA intensities is considered. However, it is possible to provide very detailed data on an individual's daily life where activity is monitored second by second. With future advances in accelerometer data processing, this risk could be increased further.

The health examinations involved in the LIV 2013 and SCAPIS study involve numerous measurements, some of which require for example blood samples that could be associated with a small health risk (51,72). The SCAPIS study also involves the use of radiation and different contrast agencies which is a potential health risk (51). On the other hand, these examinations can find pathologies and start treatment at an early stage. The data analyzed will include health variables, which are considered highly sensitive according to the European General Data Protection Regulation (GDPR) (131). This means that this data must be managed carefully to ensure confidentiality. For example, in this thesis work, health data has been stored and processed on servers with particularly high security standards. In the I.Family study specifically, the analyses had to be performed remotely through a VPN connection to a remote computer to ensure the security of the data.

Research involving sensitive personal information and physical procedures requires ethical approval (132). All studies included in the thesis have retrieved ethical approval.

- The LIV 2013 study was approved by the Regional Ethical Review Board in Stockholm (ref. 2012 / 1338-31).
- The data collection in the SCAPIS and SCAPIS pilot study was approved by the ethics committee at Umeå University (ref. 2021-228-31M) and the specific studies of the SCAPIS sample included in this thesis were approved by the regional ethical board in Gothenburg (ref. 638-16).
- The I.Family study was approved by the institutional ethical review boards of all the study centers.
 - Germany: Ethic Commission of the University of Bremen (16/01/2007 and 11/12/2012),
 - Hungary: Medical Research Council (21/06/2007, ref. 22–156/2007-1018 EKU and 18/12/2012, ref. 4536/2013 EKU),
 - Italy: Ethics Committee of the Local Health Authority (ASL) in Avellino (19/06/2007, ref. 2/CE and 18/Sep/2012, ref. 12/12),
 - Poland: Bioethical Committee of the University of Rzeszów (05/06/2013 and 01/12/2015),
 - Spain: Ethics Committee for Clinical Research of Aragon (CEICA) (20/06/2007, ref. PI07/13 and 13/02/2013, ref. PI13/0012),
 - Sweden: Regional Ethics Research Board in Gothenburg (30/07/2007, ref. 264–07 and 10/01/2013, ref. 927–12).
- The Bunkeflo study was approved by the institutional ethics committee of Lund University (ref. LU 243-01).

Despite the health and personal integrity risks involved in this thesis work, the potential benefits of improving the prevention of cardiometabolic disease are considered to outweigh the risks.

Results

Detailed analysis of physical activity intensity (1st research question)

Paper I

The association between PA and cardiometabolic health indicators was stronger with the 10 Hz method of processing accelerometer data compared to the most commonly used ActiGraph method. The explained variance was 14.2% (95% confidence interval: 13.7–14.7%) and 12.6% (12.1-13.1%) for the 10 Hz and ActiGraph methods respectively. In addition, the ActiGraph method resulted in more time spent at very vigorous intensity compared to the 10 Hz method with 1.2 and 0.4 minutes per day on average. Still, the PA pattern related to health was shifted to higher intensities with the 10 Hz method, from vigorous to very vigorous PA. In the pattern of the 10 Hz processed PA, there was more diversity between different intensities compared to the ActiGraph pattern (Figure 8).

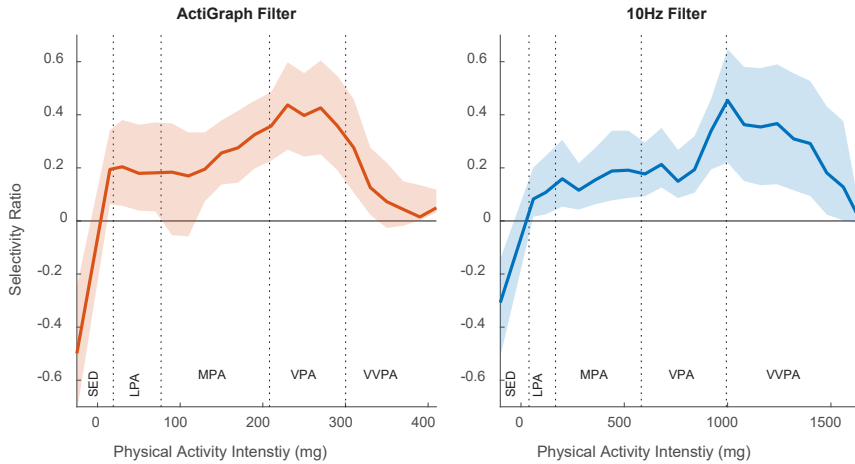


Figure 8. Physical activity patterns related to cardiometabolic health.

Selectivity ratio of the PA patterns related to cardiometabolic health based on accelerometer output from the ActiGraph method (left) and 10 Hz method (right). Shaded areas represent 95% confidence intervals. Figure based on data from Paper I.

Paper III

The PLS-DA statistical method identified statistically significant group differences in PA patterns between the intervention and control groups that have not been found before. Specifically, the intervention group spent less time around the cut-point between moderate and vigorous intensity and more time at the upper part of the vigorous intensity range. Traditional cut-points were not able to capture this difference. This is shown in Figure 9.

It was also shown that PLS-DA can be applied in paired analyses. In the paired analyses of the differences in PA patterns in the intervention group between the first and second measurements, it was found that time spent sedentary increased, and time spent at light intensity decreased. A decrease in the upper vigorous intensity range was also identified.

The PLS-DA method provides a measure of the overall effect size of the group difference represented by the proportion of correct classifications and a measure of the statistical uncertainty by a p-value retrieved from permutation tests. In addition, the differences in specific PA intensities can be visualized by selectivity ratio plots.

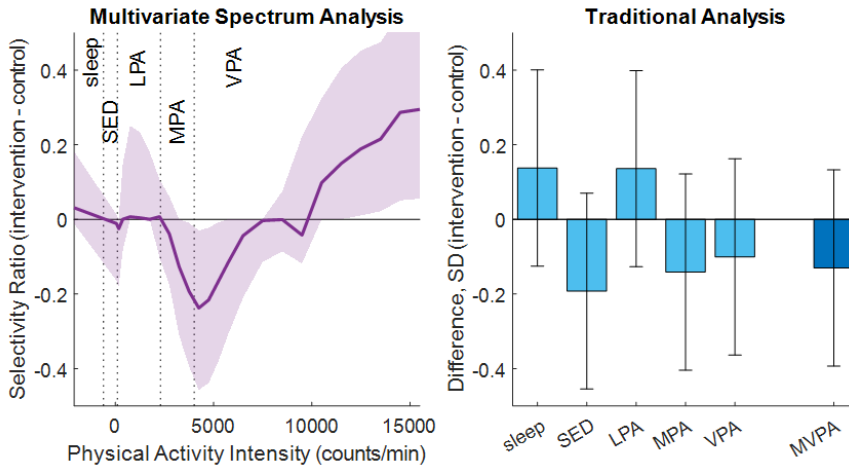


Figure 9. Group comparisons of physical activity.

Group comparisons of PA between intervention and control groups in the Bunkeflo study. The left figure shows the discriminative PA pattern from a multivariate spectrum. The right figure shows comparisons of traditional cut-points. Shaded areas and error bars represent 95% confidence intervals. Figure based on data from Paper III.

Health-related physical activity intensity (2nd research question)

Paper II

The detailed analyses of children's PA suggested that the PA pattern associated with health is different between different health variables. For example, HDL was mainly associated with PA of vigorous intensity whereas insulin resistance was more related to PA of moderate intensity as shown in Figure 10. Furthermore, MVPA was more strongly associated with health than time spent sedentary. Although the different ways of handling confounders yielded similar results, these results cannot be interpreted in the same way as in traditional regression.

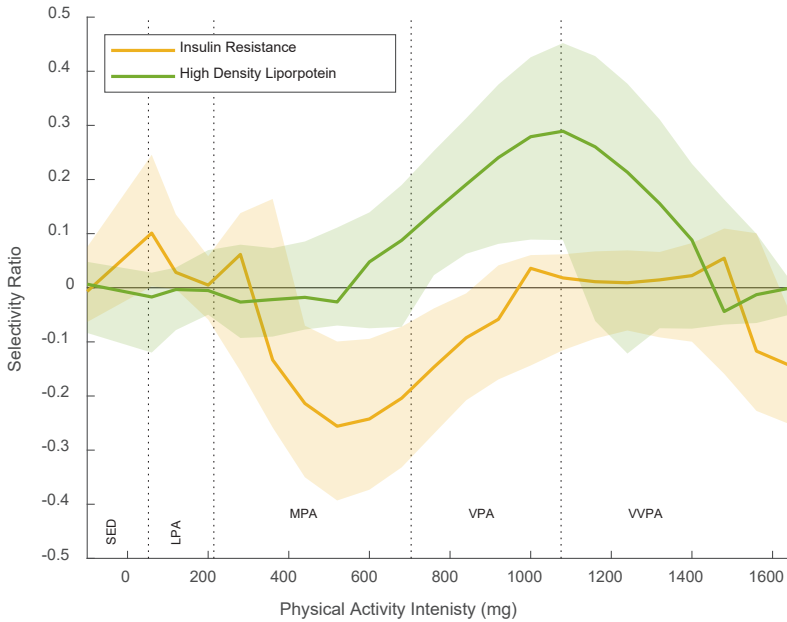


Figure 10. Physical activity patterns related to health in children.

Physical activity patterns related to HDL and insulin resistance in children. Shaded areas represent 95% confidence intervals. Higher HDL is typically considered positive for health whereas lower insulin resistance is typically considered positive for health, which explains the opposite directions of the peaks in the figure. Figure based on data from Paper II.

Paper IV and V

The same sample of middle-aged adults and similar methods of data processing and analysis were used in Paper IV and V to study the direct association between PA and cardiometabolic health. In both papers, when considering the overall sample, there were significant associations between PA and health indicators at a PA intensity corresponding to approximately 4 METs and above. Specifically, PA displayed the strongest association in the moderate intensity range for the metabolic syndrome score and in the vigorous intensity range for fitness. This is shown in Figure 11.

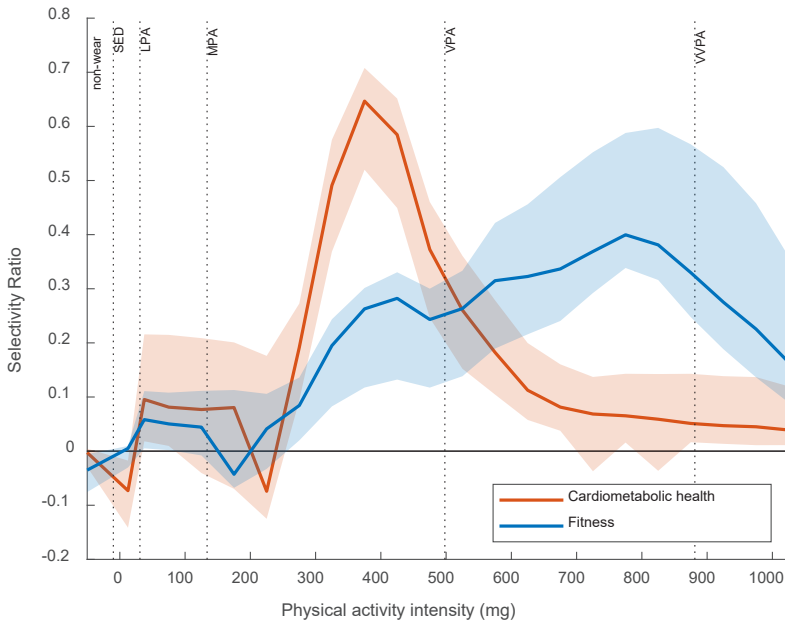


Figure 11. Physical activity patterns related to health in adults.

PA patterns related to Cardiometabolic health and fitness in middle-aged adults. Shaded areas represent 95% confidence intervals. Figure based on data from Paper V.

Physical activity, fitness, and health (3rd research question)

Paper IV

In the stratified analyses based on fitness level, the associations between absolute PA intensity and cardiometabolic health indicators were different depending on fitness level. The main associations were found in the moderate intensity range for the low fitness group, in the moderate to vigorous intensity range for the medium fitness group, and in the vigorous to very vigorous intensity range for the high fitness group. When considering relative intensity PA by relating the PA intensity to the average fitness level in each fitness group, the associations were more similar between groups. This is shown in Figure 12 by the peaks being separated for absolute intensity and overlapping for relative intensity.

Figure 12 also shows that the traditional definition of relative moderate intensity of 46% of fitness level, corresponds well with the intensity where most of the association with health is apparent. In contrast, the traditional absolute moderate intensity cut-off at 3 METs is too low for 95% of the individuals in the sample. This resulted in 99% reaching the PA recommendations based on absolute moderate intensity, compared to 21% reaching the recommendations based on relative intensity.

Based on the results that relative intensity is more representative of health-beneficial PA, individual relative intensity cut points can be calculated. For an individual with a fitness level of 35 mL./min/kg, relative moderate intensity would be approximately 4.5 METs and relative vigorous intensity would be 6.5 METs. For an individual with a fitness level of 20 mL./min/kg on the other hand, moderate intensity would be 2.5 METs and vigorous intensity would be 3.5 METs.

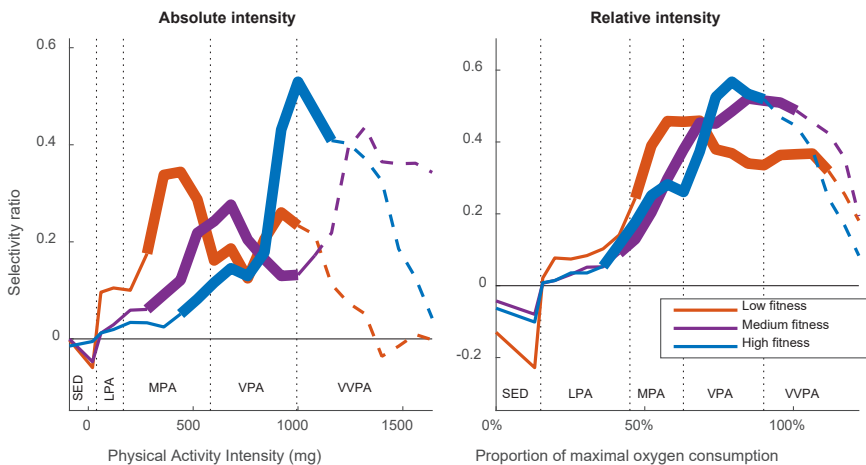


Figure 12. Association between absolute and relative intensity and fitness.

PA patterns for the association between absolute (left) and relative (right) intensity PA and fitness are shown as selectivity ratio plots. Shaded areas represent 95% confidence intervals. Figure based on data from Paper IV.

Paper V

The explained variance in the health indicators increased drastically when including sex and age as covariates in addition to PA in the PLS-SEM models, from 11% to 25% in fitness and from 14% to 38% in cardiometabolic risk factors. When including PA, fitness, and cardiometabolic risk factors in the same model the

explained variance in the health indicators increased further to 44%. In this model, fitness mediated 85% of the association between PA and the risk factors.

The standardized PA patterns of the associations, shown as multivariate correlation coefficients, differed between models. Mainly PA of moderate intensity was associated with cardiometabolic risk factors, whereas PA of vigorous intensity showed the strongest association with fitness. When including PA, fitness, and risk factors in the same model, the PA pattern of the association was more similar to the pattern related to fitness but with a peak in the moderate intensity range. The unstandardized PA patterns were more similar for all models, suggesting that one minute of more intense PA is always more strongly related to the outcomes.

PLS-SEM modeling enabled the interpretation of covariates similar to traditional multivariate regression while taking advantage of the ability of PLS to handle multicollinear variables. Although the statistical method could be considered relatively advanced, the results can easily be presented visually to facilitate interpretation (Figure 13).

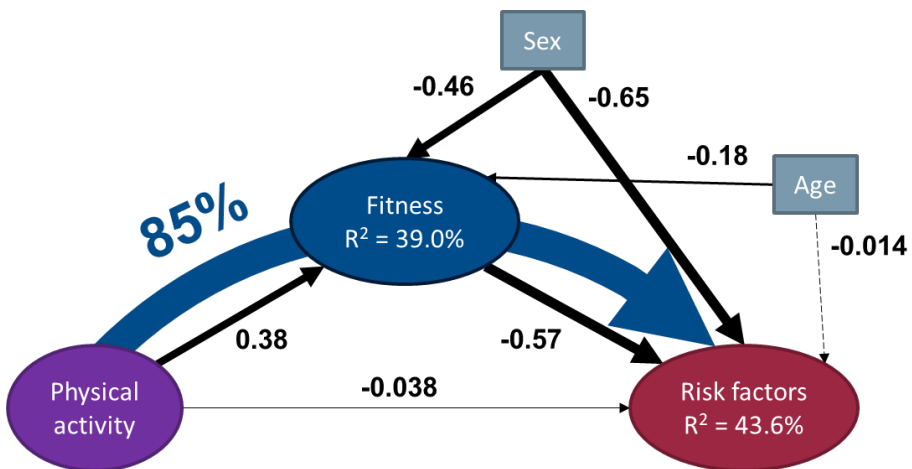


Figure 13. Mediation through fitness.

Visual presentation of the results from a PLS-SEM model of the mediation of the association between PA and cardiometabolic risk factors through fitness, while controlling for sex and age. Fitness mediates 85% of the association between Physical activity and cardiometabolic risk factors. Figure is based on data from Paper V.

Discussion

The main contribution of this thesis work is the introduction and development of multivariate statistical methods to utilize more of the information available from the measurement of PA by accelerometers. This was done in an exploratory manner to identify patterns in PA intensity that are of importance in different study designs. Thus, the main results of the thesis are not the implications in clinical or public health settings. Instead, the health-related results and clinical implications that can be deduced from the work highlight the implications of the statistical methods and support their use in future research.

Results discussion

Detailed analysis of physical activity intensity (1st research question)

Multivariate statistical analysis

The main benefit of using a detailed intensity spectrum in PA research is that the results do not depend on where an intensity cut point is set. This applies both to studies investigating associations between PA and health and to studies comparing PA patterns between groups. Paper III clearly shows that group differences in PA can be missed using traditional cut points. A part of the difference between the intervention and control group was found right at the cut-point between moderate and vigorous intensity and was therefore not captured by either of the two crude categories.

In all papers included in this dissertation, the resolution of the intensity spectrum variables was high enough to capture the most important parts of the intensity spectrum over several variables. If one intensity spectrum variable displays a completely different association compared to the neighboring variables, this could indicate that the resolution is not sufficient to capture this intensity. However, when the resolution is sufficiently high for several variables to capture the same physical behaviors, the PA variables will naturally be collinear.

Despite the high resolution of the intensity spectrum used, distinct differences between neighboring bins are apparent in many of the PA patterns of associations with health variables shown in the included papers. This suggests that there is a cut-off somewhere between the two neighboring bins that is important in the association with health. It is not possible to identify exactly at what intensity such a cut-off is located, but due to the high resolution of the spectrum, the results should give an estimate that is sufficient for further analyses. Although the resolution could be increased even further, this might require larger sample sizes and result in unstable PLS models.

It is possible to extend the spectrum to distinguish PA intensity beyond what is considered very vigorous, but the results from the papers included clearly show that the benefit of capturing PA intensity above 10 METs is limited in most cases. This is likely due to many individuals not performing any activity at these intensities and is in line with previous literature (106). In Paper I, approximately 50% of participants did not perform any activity above 10 METs. However, in Paper III, most participants performed some activity at this intensity. There could be two explanations for this. First, Paper III used the ActiGraph filter for processing the raw acceleration data, which seems to overestimate the time spent at MVPA. Second, the participants in Paper III were children, in contrast to Paper I where the participants were adults. Children often have more MVPA compared to adults, which suggests that a spectrum with higher intensities should be used for investigating them. It is also possible that studies of elite athletes might require extending the investigated spectrum further.

Accelerometer data processing

In addition to statistical methods capable of detailed analysis of PA intensity, it is important to use raw accelerometer data processing methods that can accurately capture the intensity of PA. As described in the introduction, the most commonly used method of raw data processing, ActiGraph counts, seems to have difficulties distinguishing activity in the MVPA range (67). Paper I shows that the deficits in capturing MVPA are not only apparent in lab settings, but also affect the association with health indicators in a free-living setting.

There was also a clear difference in PA volume at different intensities from the ActiGraph and 10 Hz filter. This difference is likely due to the misclassification of time in the MVPA range by the ActiGraph method (68). Although the ActiGraph method resulted in more time at moderate intensity and above, compared to the

10 Hz method, ActiGraph likely misclassified a substantial part of these intensities explaining the stronger association between PA and health with the 10 Hz method.

The misclassification by the ActiGraph method is assumed to be evenly distributed and each misclassified epoch is as likely to be misclassified as more intense as less intense. However, since individuals consistently spend less time at moderate intensity and above the misclassification results in more time at higher intensities (68). As an example, we assume a misclassification of 10% overestimation and 10% underestimation of time spent at vigorous intensity and the actual time spent at vigorous intensity is 5 minutes per day. Then, 30 seconds per day of actual vigorous intensity will be misclassified as moderate and 30 seconds will be misclassified as very vigorous. However, in an individual with 5 minutes of vigorous intensity, the average time spent at actual moderate intensity could be approximately 60 minutes per day, and for very vigorous intensity approximately 1 minute per day. The same misclassification of vigorous intensity then results in a 1% increase in moderate intensity, but a 50% increase in very vigorous intensity. The misclassification of MVPA could also explain the plateau in the PA pattern discriminating the groups in Paper III. The processing method simply cannot differentiate activities at this intensity, which has also been suggested in another study on children (133).

Comparing two different methods of accelerometer data processing is difficult since the results are highly sensitive to what cut points are used. In Paper I, cut points used were developed using the same raw data collected as a part of a calibration study, and the same methods were used for calibrating processed accelerometer data to METs. Furthermore, since the intensity spectrum was used in the PLS analysis, all information regarding health-beneficial PA is captured in the model regardless of how the traditional cut points were set. The limitations in the comparability of different measurement methods do not only apply to the measurement of intensity and volume but also to simpler measures like the number of steps per day (134).

Health-related physical activity intensity

(2nd research question)

Most of the PA patterns related to health indicators presented in the papers included in this thesis show a clear increase in the association at the absolute mid-moderate intensity range. When investigating the association with specific health indicators in Paper II, instead of a CS, there were some differences in the PA

patterns. The association with HDL was mainly found at vigorous intensity and above, whereas the association with insulin resistance was mainly found in the moderate range and decreased at higher intensities. When there is a strong association between VPA and health, this could indicate that the physiological mechanism is primarily related to the intensity of PA. When the variation in MPA explains more variation in health outcomes than variation in VPA does, this could be a sign of energy expenditure being more influential in the association with health. Previous research has suggested that moderate intensity PA might not be sufficient to increase HDL, but that vigorous intensity is required (135). In addition, previous research suggests high volumes of moderate intensity PA could improve insulin resistance compared to low volumes of vigorous intensity (136).

In Paper III and V, strong associations were found in the vigorous to very vigorous range. However, at the highest intensities where significant associations or group differences were found, individuals typically spend less than one minute per day of activity, and many have no time at this intensity. Therefore, although the intensity is very high, the contribution to total energy expenditure is very small. These standardized PA patterns, related to associations with health or group differences, therefore indicate certain behavioral patterns that may be of importance rather than a physiological mechanism directly related to the definition of PA being directly related to energy expenditure (1).

Although the strongest associations with health indicators are not always found in the vigorous range, this refers to standardized PA patterns. One minute of VPA could still be more health-beneficial than one minute of MPA. The unstandardized PA patterns presented in Paper V show that one minute of very vigorous PA has an association with the CS that is ten times stronger compared to one minute of moderate intensity. Unstandardized PA patterns from PLS models are often difficult to interpret since parts of the spectrum, either high or low intensity, might not be visible. However, it is important to understand the relative and absolute contributions of time spent at different intensities.

Physical activity, fitness, and health

(3rd research question)

The results of Paper IV and V suggest that the association between PA and health is both moderated and mediated through fitness, which is in line with what has been suggested in previous research (2,137). Specifically, the multivariate analyses

show that the moderation and mediation by fitness alter what PA intensity is associated with health.

Paper V shows that most of the association between PA and health is mediated through fitness. In addition, vigorous intensity PA was more strongly associated with health when the interplay with fitness was considered. These results suggest that PA has little direct association with health beyond the association with fitness and that fitness could be considered an indicator of health-beneficial PA. The results show that PLS-SEM is a viable tool for analyzing detailed PA intensity patterns in more complex statistical models.

The PLS-SEM method identified different patterns related to cardiometabolic health depending on whether fitness was included in the model or not. This further highlights the importance of detailed analyses of the PA intensity spectrum in different study designs. Clearly, one variable representing a fixed PA intensity (e.g. absolute MVPA) could not capture the different patterns of PA that appear depending on the structure of the statistical model. In addition, PLS-SEM can be used to analyze far more complex models with more variables, both latent and directly measured, as well as different interactions such as moderation (123).

The moderating role of fitness is shown in Paper IV by the differences in PA intensity patterns related to health indicators depending on fitness level. However, by considering PA intensity relative to fitness level instead of absolute intensity, the associations were more similar between fitness groups. This suggests that relative intensity represents health-beneficial PA better since it is generally applicable regardless of fitness level. It has previously been shown that fitness could be considered a moderator of the association between PA and health (138), but the associations of different intensity levels have not been studied before.

The intensity where absolute and relative intensity PA is equal is different depending on fitness level. This means that although the absolute 3 MET's cut-off was too low in the SCAPIS sample, it might be suitable for elderly individuals or individuals with medical conditions. However, the absolute 6 and 9 MET's cut-offs do correspond with the relative intensity cut-offs at 64 and 91% in the SCAPIS sample (23). Therefore, in a sample where 3 MET's would be an appropriate moderate cut-off, 6 and 9 MET's would be too high cut-offs for vigorous and very vigorous, respectively. Hence, the traditional absolute intensity cut points are not congruent.

The results of Paper IV suggest that a single variable representing MVPA relative to a representative fitness level could be used to capture most of the association between PA and health variables. However, these results could not

have been acquired without the use of multivariate analyses of the PA intensity spectrum. An exploratory approach was used when analyzing associations with health from absolute and relative intensity. Hence, the analyses were not based on any prior assumptions on what intensities would be most important. Yet, when inspecting the resulting PA intensity patterns, the results agreed with the relative intensity cut-offs proposed by previous literature (23).

Although no studies have investigated the association between accelerometer-measured relative PA intensity and health indicators previously, it is not particularly complicated to adjust cut points using published calibration equations. Paper IV includes calibration equations for determining relative intensity cut-offs for the 10Hz FEM method, and previously published calibration studies also include equations that can be used (101,139). However, measurements of fitness level in large-scale observational studies are not as common as accelerometer measurements. As an alternative, the fitness level of a sample could be estimated based on age, sex, and self-perceived physical capacity (140).

Health-related implications

General recommendations on PA suggest PA of at least moderate intensity is required for most health benefits (7). In line with the recommendations, the results in this thesis suggest there is a specific threshold in the moderate intensity range above which most of the health benefits are acquired. However, this threshold seems to be closer to 4.5 METs instead of the absolute moderate cut-off of 3 METs most often used in PA research (2). On the contrary, the definition of moderate intensity relative to fitness level (46% of maximal oxygen consumption) corresponds well with where the health benefits are apparent, regardless of fitness level (23).

In the first general recommendations on moderate intensity PA presented in the Surgeon General report (27), the definition of absolute moderate intensity is discussed. The report states that absolute moderate intensity would be above 4.5 for middle-aged adults (40-64 years) and above 3.6 for old adults (65-79 years). However, in the conclusions regarding a general definition of absolute moderate intensity, 3 METs is suggested. The rationale for using a 3 METs cut-off remains unclear, but this conclusion has certainly had a vast impact on the PA research field.

Perhaps the most clinically important, and at the same time simple, implication of the results in this thesis is that the traditional moderate cut of at 3 METs is too

low for most individuals. Since multivariate analysis of PA intensity patterns can be difficult to both conduct and interpret, this might not always be the best option in all studies. However, adjusting the moderate intensity cut-off to 4.5 METs should be considered in most studies, except when studying individuals with particularly low fitness levels e.g., heart failure patients. When studying individuals with particularly low, or high, fitness levels, PA intensity should be analyzed relative to the average fitness level in the sample. This is emphasized by the results suggesting that PA should be at a sufficiently high intensity to improve fitness.

Although relative intensity represents health-beneficial PA regardless of fitness level, it should not be used in cross-sectional research relative to individual fitness. If doing so, individuals with low fitness are typically more active than individuals with high fitness,(141) which is also shown in Paper IV. The use of individual relative intensity is more suitable for use in longitudinal studies. In longitudinal studies, individuals who are more active from a relative point of view are more likely to improve their health over time. Individuals with high fitness, however, may already have benefited from most health improvements due to their high PA and high fitness. This also emphasizes how PA should be progressed over time. Individuals with low fitness could start with PA of absolute light intensity (which could still be relative moderate intensity) and improve their fitness level. Once fitness has been improved, the intensity of PA should be progressed to achieve further improvements.

Measurement of PA is highly sensitive to what processing methods are used. Defining who is considered sufficiently physically active will always have to be relative to the specific measurement methods used for determining what level is sufficient in the first place. Paper IV clearly shows that the degree of fulfillment of PA recommendations varies vastly depending on how the moderate intensity threshold is defined. However, this refers to recommendations on PA volume and intensity that are mainly based on self-report data. Like with different methods of accelerometer data processing, self-reported PA differs from other methods of estimating PA level (41,43). Although self-reported PA displays substantially weaker associations with health indicators compared to accelerometer-measured PA (42,43), self-report is likely a more appropriate method to use for determining fulfillment of PA recommendations.

Limitations

All the papers investigating the association between PA and health indicators included in this thesis are based on cross-sectional data. This implies that no conclusions regarding causality can be made. Although the common view in PA research is that increased PA improves health (2), the opposite could also be true, that low PA rather is caused by poor health and inability to be active. For example, there are studies suggesting that increased weight during childhood leads to lower levels of PA, rather than low levels of PA leading to increased weight (142,143). However, there is strong evidence from intervention studies that PA increases health in the short term (2).

The cross-sectional nature of the data also prevents proper analysis of the mediating role of fitness in the association between PA and health in Paper V (144). To prove that fitness mediates the health benefits of PA, multiple measurements of PA, fitness, and health must be conducted and the difference between measurements must be considered. However, regardless of whether fitness mediates the effect of PA on health, the interconnected associations between the three are important to understand.

In addition to the cross-sectional study designs, the studies are observational. Observational studies are limited to studying the associations apparent in the sample studied. Hence, the results cannot be extrapolated beyond normal PA patterns. This means, for example, that the results should not be interpreted about extremely high volumes of very vigorous intensity PA, apparent in elite athletes. Furthermore, this is a limitation in the interpretation of the different PA patterns in the fitness groups in Paper IV. The shifted PA patterns related to health indicators may be because of the low fitness group not being active at vigorous intensity, and therefore no association could be apparent. To properly test if there is a difference in physiological response to absolute and relative intensity depending on fitness level, randomized controlled trials must be performed.

The advancements related to analyzing objectively measured PA presented in this thesis work are suitable for investigating associations with health indicators and when comparing groups. However, with the current state of PA research, it is not suitable to use the methods of data processing and statistical analyses for descriptive purposes alone. The results from previous research have been produced with different methods that are not comparable to the methods used in this thesis work. Therefore, volume and intensity cannot be compared to previous studies.

The volumes of PA in the papers included in this thesis are generally higher than in most other studies. For example, 99% of individuals fulfilled the recommendations in Paper IV. The methods of these papers include short epoch lengths and triaxial accelerometer data, which both result in higher volumes than longer epoch lengths and accelerometer data from the vertical axis only (61,145). In addition, the SCAPIS sample seems to be more active than many other large-scale study samples from an international point of view (145). This geographic difference, with more PA in Sweden, was also found in Paper II. However, this cannot fully compensate for the high volumes. The high volumes could also be explained by the association between accelerometer output processed by the wider frequency filters and oxygen consumption. Most previous calibration studies for translating accelerometer output to energy expenditure used linear calibration (101,139), whereas the calibration used in the papers in this thesis used smoothing splines which allow for non-linear associations (68). Yet, the association between mechanical work and oxygen consumption seems to be non-linear (67).

All the analyses performed as a part of this thesis use an explorative approach where the focus is on the external validity of the PA measurement. First, exploratory means that the results regarding PA intensity explore what intensities are primarily related to different outcomes, rather than confirming that a particular intensity is most important. Results based on exploratory analyses are considered more uncertain than their confirmatory counterpart (146). Second, external validity refers to the degree that which the PA measurement captures information that is directly related to health outcomes. The health benefits of PA are mainly thought to be related to the energy expenditure from PA (2). Therefore, PA measurement by accelerometry has traditionally been calibrated to estimate energy expenditure, and associations between PA and health have been interpreted regarding this energy expenditure. The analyses in this thesis investigate the association between accelerometer-measured PA intensity and health outcomes directly, without first translating accelerometer data to energy expenditure. Hence, this thesis does not focus on the physiological mechanisms of the association between PA and health outcomes, but instead on the information available from the accelerometer measurements.

Future research

The papers included in this thesis have shown that the most used methods for processing and analyzing accelerometer data in PA research can be improved. Still,

a more thorough investigation of optimal processing methods for accelerometer data is warranted. Improvement of accelerometer data processing methods should consider accuracy from a biomechanical and physiological point of view. In addition, the methods must also be able to specifically capture PA that is related to health benefits, which is why the association with health could be just as important as accurate measurement from a measurement methods perspective. Furthermore, processing methods must be feasible in terms of computational resources, etc.

Although some specific improvements in accelerometer data processing have been suggested in this thesis, these improvements are still difficult to implement for most researchers. The technical specifications of the algorithms are described in detail in the published studies, but this requires specific signal-processing knowledge to implement in research. Future work should facilitate the use of these improved methods by making them accessible to all researchers who could benefit. Currently, the most widely used open-source accelerometer data processing tool is the GGIR package (95), which could be a viable option for implementing the improved processing methods in.

The results presented in this thesis clearly show the importance of specific intensities of PA. In addition to this, there are methods for activity class estimation (55,147). The main advantage of using processing methods for activity classification is the facilitation of the interpretation of activity type. This enables identifying bicycling as well as differentiating sitting from standing still (55), which could be an important addition to the measures of PA intensity. Future studies should incorporate measures of PA intensity and activity type in the same statistical models to benefit from more detailed information. However, variables representing some activity types are expected to be highly collinear with some of the PA intensity variables. Since multivariate statistical methods are well-suitable for analyzing multicollinear data, these methods could be used to combine measures of activity type and intensity.

There is increasing interest in artificial intelligence and machine learning approaches to accelerometer data analysis (39). However, the advantages of these methods compared to traditional accelerometer data processing are unclear (102). In the next decade, however, these methods will likely improve drastically and could revolutionize the PA research field.

Conclusions

The results of the papers included in this thesis highlight the importance of detailed analyses of PA intensity measured by accelerometry. Using a wider frequency filter than the traditional 1.63 filter in the processing of raw accelerometer data results in stronger associations with health indicators and allows for a more detailed interpretation of PA intensity. The PA intensity pattern related to health is different for different health indicators and different populations. Fitness level determines the PA intensity required for associations with health and the main health benefits come from the PA that improve fitness. Interpretation of PA intensity patterns requires nuance and consideration of the interplay between several factors. Detailed analysis of PA patterns using multivariate statistical methods captures these nuances and provides novel insights regarding the importance of specific PA intensities in different study designs.

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Physical activity is widely recognized as an important factor for health. Yet, the health implications of specific physical activity intensities and the intricate role of fitness in the relationship between physical activity and health remain less clear. While accelerometers provide objective measurements of physical activity intensity, established methods for data processing and statistical analysis often underutilize this information. This doctoral thesis introduces and further develops multivariate statistical methods to analyze accelerometer-measured physical activity intensity with more detail.

Data from four separate studies were reanalyzed using improved accelerometer data processing methods and multivariate statistical approaches. The results highlight the importance of detailed analyses of physical activity intensity. The patterns of physical activity intensity relating to health were different for different health indicators and different groups. Furthermore, fitness level determined the physical activity intensity required for associations with health. Analysis of physical activity using multivariate statistical methods captures more detail in the accelerometer data and enables studying the complex role of physical activity intensity in different study designs.



Jonatan Fridolfson is a physiotherapist and sports scientist with expertise in technical analysis of accelerometer-measured physical activity.



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