DISSERTATION FROM THE
NORWEGIAN SCHOOL OF
SPORT SCIENCES
2020

Mette Stavnsbo

Cardiometabolic risk factors in children

Reference values, association with cardiorespiratory fitness and effects of the Active Smarter Kids (ASK) physical activity intervention

NIH NORWEGIAN SCHOOL OF SPORT SCIENCES

nih.no

Mette Stavnsbo

Cardiometabolic risk factors in children

Reference values, association with cardiorespiratory fitness and effects of the Active Smarter Kids (ASK) physical activity intervention

DISSERTATION FROM THE NORWEGIAN SCHOOL OF SPORT SCIENCES • 2020

ISBN 978-82-502-0575-8

English summary

Background: Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality globally. Although CVD is not manifest in children, studies have shown that the pathophysiological processes of atherosclerosis starts in childhood and progress throughout life, with the acceleration highly depending on lifestyle. Thus, surveillance of the development of CVD (or cardiometabolic) risk in children, as well as the development of preventive and health promotion strategies, are important from a public health perspective.

Aims: First, to produce international reference values for cardiometabolic risk factors in children and adolescents, to provide a basis for comparison between studies when using continuous cardiometabolic risk scores. Second, to investigate cardiometabolic risk levels in Norwegian children compared to the international reference values, as well as the association between cardiorespiratory fitness (CRF) and the reference-standardized clustered risk score. Last, to evaluate the effect of the Active Smarter Kids (ASK) daily physical activity (PA) intervention on single and clustered cardiometabolic risk factors.

Methods: Reference values were based on pooled data from cohorts of children sampled from different parts of Europe (North, South, Mid and Eastern) and from the United States. In total, 22,479 observations (48.7% European vs. 51.3% American) from children aged 6–18 years, were included in the study. Two studies were based on data from the ASK study, a cluster-randomized controlled trial including 1,129 fifth-grade children from 57 schools in Western Norway, conducted between November 2014 and June 2015. The clustered risk scores consisted of waist circumference (WC), systolic blood pressure (SBP), total cholesterol (TC):high-density lipoprotein (HDL)-ratio, triglyceride (TG), homeostatic model assessment (HOMA)-score, with and without CRF. The Andersen test was used to measure CRF and PA levels were measured objectively by accelerometry (GT3X+).

Results: Reference values were produced for 14 of the most commonly used cardiometabolic risk factors in pediatric clustered risk scores and presented by age and sex. Log-transformed reference values were also provided for skewed variables. The clustered risk score was higher in the Norwegian children, but decreased to below international levels when CRF was including. CRF had a significant inverse association with the clustered risk score (excluding CRF). The ASK intervention had no significant influence on single or clustered cardiometabolic risk factors in the total sample. However, in children with the most unfavorable cardiometabolic baseline values, beneficial effects were found on SBP, TC:HDL-c ratio and the clustered cardiometabolic risk score. The effect of the intervention

was also moderated by sex, and girls had a greater effect of the intervention on WC and CRF than boys.

Conclusion: The international reference values suggest a common standard to define cardiometabolic risk in children and adolescents, and allow for comparison of continuous clustered risk scores. Norwegian children have substantially higher CRF levels than international standards, and including CRF in clustered risk scores reduces overall risk to below that of international levels. CRF is associated with improved cardiometabolic health in children. No effects of the ASK school-based PA intervention on cardiometabolic risk were found in the total sample. However, girls and children with the most unfavorable risk factor levels can benefit from school-based PA interventions.

Key words: cardiometabolic risk, clustering, standardization, cardiorespiratory fitness, physical activity, intervention, children, adolescent.

Norsk sammendrag

Bakgrunn: Hjerte og karsykdom (HKS) er den ledende årsaken til morbiditet og mortalitet globalt. Til tross for at HKS ikke er manifest i barn, starter den aterosklerose prosessen allerede i barndommen og videreutvikles gjennom livet, hvor akselerasjonen avhenger av livsstil. Fra et folkehelseperspektiv er det derfor viktig å overvåke utviklingen av risiko for framtidig HKS blant barn og unge, på lik linje med utviklingen av forebyggende og helsefremmende strategier.

Formål: For det første, å utvikle internasjonale referanseverdier for HKS risikofaktorer for barn og unge, for å tilby et sammenligningsgrunnlag når kontinuerlige HKS risikoskårer benyttes. For det andre, å undersøke nivået i individuelle og klyngede (clustered) HKS risikofaktorer blant norske barn sammenliknet med internasjonale referanseverdier, samt undersøke assosiasjonen mellom fysisk form og den referanse-standardiserte *clustered* risikosskåren. For det tredje, å vurdere effekten av Active Smarter Kids (ASK) studiens intervensjon med fysisk aktivitet på både individuelle og *clustered* HKS risikofaktorer.

Metode: Referanseverdiene er basert på data fra flere kohorter av barn fra Europa (Nord-, Sør-, Midt- og Øst) og Nord-Amerika. Totalt ble 22479 observasjoner (48,7% europeiske og 51,3% amerikanske) fra barn og unge i alderen 6 til 18 år inkludert. To studier ble basert på data fra ASK-studien, en skolebasert *cluster*-randomisert kontrollert intervensjon som inkluderte 1129 femteklasse elever fra 57 skoler i Sogn og Fjordane, gjennomført mellom november 2014 og juni 2015. HKS risikoskåren var basert på midjemål, systolisk blodtrykk (SBP), total kolesterol:høy-densitet lipoprotein kolesterol (TC:HDL-c ratio), triglyserider (TG), homeostatic model assessment (HOMA) score, med og uten fysisk form. Andersen testen ble brukt til å måle fysisk form og fysisk aktivitet ble målt objektivt med akselerometer (GTX3+).

Resultater: Referanseverdier for alder og kjønn ble etablert for 14 vanlig benyttet HKS risikofaktorer. Log-transformerte referanseverdier ble rapportert for ikke-normalfordelte variabler. Norske barn hadde en høyere *clustered* risikoskåre enn de internasjonale referanse barna, men når fysisk form ble inkludert falt risikoskåren til under det internasjonale nivået. Fysisk form hadde en invers assosiasjon med den *clustered* risikoskåren (uten fysisk form). ASK intervensjonen hadde ingen signifikant effekt på verken de individuelle eller *clustered* HKS risikofaktorer i den samlede populasjonen. ASK intervensjonen viste imidlertid positive effekter på SBT, TC:HDL-ratio og den *clustered* risikoskåren hos de barna som hadde de minst gunstige HKS verdiene i utgangspunktet. Effekten av intervensjonen ble også moderert av kjønn, og jentene opplevde større effekt på midjemål og fysisk form sammenliknet med gutter.

Konklusjon: De internasjonale referanseverdiene forslår en felles standard for å definere HKS risiko blant barn og unge og muliggjør sammenligning av kontinuerlige *clustered* risikoskårs. Norske barn har markant høyere fysisk form enn internasjonale standarder, og en *clustered* risikoskår inklusiv fysisk form reduserer den overordnede risikoen til under det internasjonale nivået. Det var ingen signifikant effekt av ASK-studien på risikofaktorer for HKS i den samlede populasjonen. Analyser av sub-grupper viste imidlertid at både jenter og barna som hadde de minst gunstige HKS verdiene i utgangspunktet hadde en positiv effekt av den skolebaserte intervensjon med fysisk aktivitet.

Nøkkelord: risiko for hjerte- og karsykdom, klynge, standardisering, fysisk form, fysisk aktivitet, intervensjon, barn, ungdom.

Acknowledgements

This thesis is a part of the Active Smarter Kids (ASK) study, which was carried out at the Western Norway University of Applied Sciences (HVL) in collaboration with the Norwegian School of Sports Sciences (NSSS). The ASK project was funded by The Research Council of Norway (grant number 221047/F40 and 249932/F20), the Gjensidige Foundation (grant number 1042294), HVL and NSSS.

I would like to express my utmost gratitude to my main supervisor and ASK project coordinator – Professor Geir Kåre Resaland – also known as the "Man with the plan" or simply, GK! GK, you have been more than a supervisor to me during this long journey – you are also my mentor, a personal mind-booster and a friend. Thanks for always having time for me – even when *you don't*, and for always believing in me – especially when *I don`t*! You are a BIG man with a BIG personality – and you also have a BIG place in my heart! Your pracademic work and your devotion to make childhood a more happy and healthy time in life is inspirational. GK – you are simply fantastic and I will never forget the time I have had under your BIG caring wings.

To my two co-supervisors - ASK project leader and Head of the Department of Sports Medicine at NSSS, Professor Sigmund A. Anderssen and leader of the ASK international advisory board, Professor Lars B. Andersen - it has been a true privilege to have two such competent, experienced and wise professors on my team. You are absolutely rock-stars! Although you have not been personally present daily at HVL, I have always felt you close by. Sigmund, I really appreciate all the times you have called – just to hear how Mette was doing in Sogndal. Lars, first my teacher at the University of Southern Denmark, then my PhD supervisor and now colleague at HVL. What a journey! A big warm thanks to you and your sweet wife, Birgitte, for the times you have visited us privately. Thank you both, Sigmund and Lars, for all your good advices, critical but righteous comments and big support during the years.

Eivind Aadland – although not supervisor on the paper, you have earned the credit as one. I still get stunned by how thorough you are and yet, so quick! You make the most complicated things seem SO easy. You are truly talented! Thank you for your patience with me and for guiding me safely through these years of statistical challenges. You have a ticket, personally from me, directly into heaven. Also a grateful thanks to my many other fantastic co-authors for the great effort on the articles.

Thanks to Mai Chin A Paw, University Research Chair professor at VU University Medical Center in Amsterdam, where I had the pleasure of having my academic stay abroad. Thank you for your positive reception, help and support before and during my stay, as well as your sharp critical eyes on my papers. The time went past so quickly, but my family and I enjoyed every moment in Amsterdam – and at least we reached our picnic! Also a warm thanks to Eline Vos, your personal "handy-woman", secretary and junior researcher at VUmc, for including me socially at VUmc and making my entry to the university so smooth.

To Turid, Katrine and Laura – my fellow PhD students in the ASK study - thank you for all your support, personal talks and peptalks throughout the years. You are a bunch of “power ladies” and I’m truly grateful to have had you at my side during this process. We started being at very different places in our lives, but “adopted” a common child – ASK. As with any other child, we have been frustrated, sleepless and have had a lot of concerns regarding “his” welfare… but indeed also proud when we saw the first achievements and when he started to smile back at us. Thank you so much for the “fight”, many unforgettable years, and for your much appreciated friendship.

First research assistant and now PhD student in the ASK study - Øystein Lerum - the man who gets everything done with a smile. Thanks for all your help and support during the data collection period, for your warm personality and good humor. I also wish to send a general thanks to my many other fantastic colleagues at HVL. A special thanks goes to the “girl group” of current PhD students, Ada, Solveig, and Ellen, for your support and encouragement. Also, a special and personal thanks to the current Head of Department, Frode Fretland and Assistant Head of Department, Ane Solbraa, for your excellent leadership, understanding and flexibility during my time as a PhD student.

To my very best “old” friends – Nadia, Anita, Lea and Trine, and to my best Norwegian friend, Ida-Beate, thank you for being so fantastic! I’m truly thankful to call such beautiful, funny, crazy and caring personalities for my friends! Thanks for your endless support and encouragement, as well as your patience with me. You are my sisters in heart and soul.

To my parents, thanks for raising me up being “my own”, stimulating my stubbornness and will to follow my heart, created from a childhood based on a mix of “hippie” and discipline. Thanks for your indispensable support during this long university journey – both economically and personally. To my sister, Ida, you are the definition of *sweet and good* and a personal inspiration. I love you all deeply.

Smilla Pi and Nord Huxi, my two AMAZING little ones - you fill my life with love, laughter and joy. You (and your father) are the foundation of my life and no matter where I am, you are always in my thoughts. Being your mum makes me endlessly proud and I love you from the bottom of my heart – to the moon and back!

Finally, my wonderful husband and power dad – Anders! Your devotion to both the children and me is endless, and as with our children, your patience during the years has been more than I could ask for. I’m forever grateful for having you in my life. You are the one and only – now and forever!

Mette Stavnsbo
July 2019, Sogndal

Abbreviations

ASK	Active Smarter Kids
BMI	body mass index
CI	confidence interval
Cluster-RCT	cluster-randomized controlled trial
cpm	counts per minute
CRF	cardiorespiratory fitness
DBP	diastolic blood pressure
HDL-c	high-density lipoprotein cholesterol
HOMA	homeostatic model assessment
ICC	intra-class correlation
IQR	interquartile range
LDL-c	low-density lipoprotein cholesterol
MVPA	moderate-to-vigorous physical activity
PA	physical activity
PE	physical education
SBP	systolic blood pressure
SD	standard deviation
Sum4Skin	sum of four skinfolds
TC	total cholesterol
TG	triglycerides
VPA	vigorous physical activity
WC	waist circumference

List of papers

This dissertation is based on the following original research papers, which are referred to in the text by their Roman numerals:

Paper I

Reference values for cardiometabolic risk scores in children and adolescents – suggesting a common standard

Paper II

Cardiometabolic risk factor levels in Norwegian children compared to international reference values: The ASK study

Paper III

Effects of the Active Smarter Kids (ASK) physical activity intervention on cardiometabolic risk factors in children: a cluster-randomized controlled trial

List of tables

List of figures

Table of content

Introduction

Cardiovascular disease (CVD) is the leading cause of disease-related morbidity and global mortality among adults (1, 2). In children, a clinical manifestation of CVD is not present, but studies have shown that atherosclerosis originates during childhood (3-7). In addition, studies suggest that individual CVD (or cardiometabolic) risk factors, such as obesity (8), blood pressure (BP) (9), and lipids/lipoproteins (10) as well as the clustering of these risk factors (11-14), may track from childhood to adulthood, conveying increased risk of CVDs and type 2 diabetes. Thus, monitoring, surveillance and profiling of clustered cardiometabolic risk in children, as well as the development of effective prevention and health promotion strategies, is important from a public health perspective.

The term metabolic syndrome refers to clustering of cardiometabolic risk factors and is diagnosed when individuals' exhibit elevated levels in three or more risk factors above certain thresholds (15-18). However, no consensus exits as to which exact risk factors and thresholds should be used to define the metabolic syndrome (15-21). Furthermore, dichotomization of risk variables ignores that cardiometabolic risk likely is a continuum and substantially decreases the amount of available information, therefore also the power of statistical analysis (22-24). In addition, since children do not have established CVDs, makes the argument to use thresholds in children even more unsustainable. Thus, the use of continuous clustered risk scores is arguably superior to dichotomized versions, especially in the pediatric population. From this perspective, international reference values is highly warranted to offer a common standard for defining levels of cardiometabolic risk in children and adolescents, when using continuous clustered risk scores.

Physical inactivity and low cardiorespiratory fitness (CRF) are major causes of the cardiometabolic health burdens worldwide (25, 26). The school has been emphasized as an ideal environment for public health interventions by the World Health Organization (WHO) among others (27, 28), since children spend a substantial fraction of their waking hours in school. Furthermore, as an institution, the school has the means to reach most children irrespective of socioeconomic status or parents' attitude and behavior regarding PA.

The aim of this thesis was threefold. The *first aim* was to produce international reference values for cardiometabolic risk variables in children and adolescents. The *second aim* was to investigate and compare cardiometabolic risk levels in 10-year-old Norwegian children from the Active Smarter Kids (ASK) study to the international reference values and examine the association between CRF and the reference-standardized clustered risk score. The *third aim* was to investigate the effect of the ASK study's school-based PA intervention on single and clustered cardiometabolic risk in children.

Cardiometabolic risk factors and clustering

Cardiometabolic risk refers to increased risk of type 2 diabetes and cardiovascular diseases (CVDs). The prevalence and incidence of type 2 diabetes has increased markedly worldwide during the lasts three decades (29). Type 2 diabetes is characterized by insulin resistance and beta-cell dysfunction, which causes hyperglycemia (30). Besides being a risk factor for CVDs, the long-term complications of type 2 diabetes include nervous system damage (neuropathy), kidney disease (nephropathy), and eye damage (retinopathy) (31). Cardiovascular diseases are the leading cause of death globally (1, 2). Cardiovascular disease is mainly caused by advanced atherosclerosis and is characterized by conditions affecting the heart or blood vessels, with coronary heart disease (CHD) accounting for most CVD deaths, followed by stroke and heart failure (32).

In children, the risk of CVDs is more difficult to define than in adults, since no manifest disease (or death from these) have yet occurred. Although the clinical manifestation of CVDs often do not occur before middle-age, studies have shown that the atherosclerosis process starts during childhood and progresses throughout life (3, 33, 34). The atherosclerosis process, in short, appears to be initiated by lipid retention (in the arterial wall), oxidation, and modification, which incite chronic inflammation, and ultimately might cause ruptures, thrombosis occlusion or stenosis (35). In morphological studies, the progression of atherosclerosis with age has been shown to be dependent on several cardiometabolic risk factors. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study has provided strong evidence that atherosclerotic changes are related to obesity, hypertension, dyslipidemia (decreased high-density lipoprotein cholesterol (HDL-c) and increased non-HDL-c), and insulin resistance, by studying aorta and coronary arteries in approximately 3,000 autopsied individuals (3-7). The Bogalusa study, which investigated the degree of atherosclerosis in coronary arteries and levels of cardiometabolic risk factors in young patients 2-39 years of age, has provided similar results (34). The Bogalusa study also found that the extent of atherosclerosis increased markedly with the number of cardiometabolic risk factors independent of age. Individuals with 0, 1, 2, or 4 risk factors had 1.3%, 2.5%, 7.9%, or 11%, respectively, of the arterial wall covered with fatty streaks (the earliest visible lesion of atherosclerosis) (34).

The term *clustering* is used to describe the physiological phenomenon where several risk factors are not independently distributed in the same individual, and where cardiometabolic risk refers to those individuals having clustering in the upper range (36, 37). The clustering of obesity, hypertension, dyslipidemi and insulin resistance are the risk factors most acknowledge to constitute the metabolic syndrome (16, 18, 19). The clustering of these risk factors appears to be caused by shared underlying processes in the pathophysiology of CVD and type 2 diabetes (38), related to oxidative stress,

systematic inflammation and adipocyte dysfuntion (39-42). However, these processes are not fully understood. Since most single risk factors pose a low immediate risk to children, clustered risk factors are considered a more valid method to assess cardiometabolic risk in children and provide a more fully picture of general health (36). Furthermore, single risk factors have several disadvantages, such as being sensitive to day-to-day fluctuations (36), and in general have shown a weaker association with CVD outcomes in adulthood than clustered risk scores (11-14).

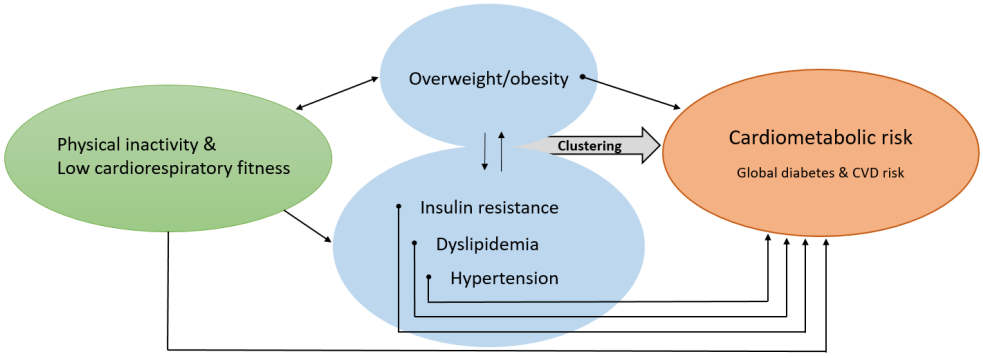

Figure 1. Physiological pathways and risk factors contributing to cardiometabolic risk (figure modified from the Consensus statement from the American Diabetes Association and the American College of Cardiology Foundation (Brunzell et al. (43)).

Although the pathophysiology of cardiometabolic risk is not fully elucidated, it has been suggested that obesity and/or insulin resistance play a pivotal role (44, 45). However, it is difficult to determine which risk factor in the metabolic syndrome is the most dominant, since these risk factors are strongly interrelated (43). Cardiometabolic risk is highly heritable and also influenced by other non-modifiable factors such as age, sex and ethnicity (46). However, cardiometabolic risk is modifiable by lifestyle, such as levels of PA and CRF (43, 47, 48), which underpins the opportunity to interrupt these processes with lifestyle modification (see **Figure 1**).

As in adults, the incidence of type 2 diabetes among children and adolescents has increased significantly in recent years (49), which in general is considered to be a consequence of an inactive lifestyle and unhealthy food habits. Studies have shown that clustered cardiometabolic risk scores in children predicts the early onset of type 2 diabetes (50). In addition, clustered cardiometabolic risk in childhood may track into adult life and is linked to type 2 diabetes and CVD in adulthood (11-14, 51). This indicates that measuring risk factors at an early stage is important from a public health

perspective and provides essential knowledge regarding the need for implementation of preventative cardiometabolic disease strategies. However, several pitfalls exist when comparing clustered risk scores in children and adolescents. Among the most serious pitfalls are selection bias leading to low prevalence and misclassification of individuals, weighting of risk factors, and sample-specific risk scores, all of which make the comparability of clustered risk between populations difficult.

The need for a common standard to define cardiometabolic risk

The first time clustering of CVD risk factors were classified as a syndrome in adults, was in 1923 by Kylin (52). Albeit, it was not until Reaven's Banting Medal award lecture in 1988 that the pathophysiological condition “Syndrome X” (45), which grounded the later metabolic syndrome, was unified and gained considerable foothold in the medical literature. A 2005 Medline search for the keywords *metabolic syndrome*, *syndrome X* and *insulin resistance syndrome* by Kahn et al. (53) recognized 4,646 citations, 3,948 of which reported on studies conducted in humans (date: 28 January 2005). Today, approximately 15 years later, this number has increased to 54,054 studies conducted in humans, out of 69,321 citations (date: 28 February 2019), using the same search conditions as Kahn and colleagues. Despite an increased interest in the metabolic syndrome and efforts of harmonizing the syndrome (15), limited consensus exists regarding its components and use. Several researchers and organizations have proposed a number of different definitions of the syndrome. Some of the most used are the definitions suggested by the WHO (16), the US National Cholesterol Education Program’s Adult Treatment Panel III (NCEP ATP III) (17), and the International Diabetes Federation (IDF) (18). These definitions are all based on dichotomized variables, and the metabolic syndrome is diagnosed when an individual exhibits elevated levels in three or more risk factors above certain thresholds. These thresholds, however, differ among the definitions. In addition, different criteria’s and single risk components are used to construct the metabolic syndrome, as well as weighting different single risk factors over others. For instance, the definition by the WHO weights impaired glucose tolerance (16) in their definition, while IDF weights central obesity (18). In somewhat adjusted versions, these definitions have also been suggested to account for children and adolescents (19-21). Divergent definitions of the metabolic syndrome not only make comparison between studies difficult in general, but the arbitrarily adjusted versions also pose a major limitation when estimating prevalence and trends of cardiometabolic risk in children. Studies comparing the prevalence of the metabolic syndrome in the same group of children using different definitions, show large variations. For instance, a study by Reinehr et al. (2007) investigated the prevalence of the metabolic syndrome using eight different definitions, including the WHO, NCEP

ATP III, and IDF definitions, in the same group of 6-to 16-year-old children. The study found that the prevalence varied between 6% and 39% depending on the definition used (54). In addition, only 2% of the children fulfilled the criteria the metabolic syndrome across all definitions, indicating a low degree of overlap among the different proposed definitions (54). Thus, it is not meaningful to compare across studies that use diverse definitions of the metabolic syndrome. Despite evidence associating the commonly applied definitions of the metabolic syndrome to CVDs (55, 56), the use of dichotomized variables substantially decreases the available information and power of statistical analyses and ignores that cardiometabolic risk is likely a continuum. In addition, children do not yet have established CVDs, which further makes the argument for using thresholds in children inadequate. Thus, the use of a continuous cluster risk score in children is arguably superior to a dichotomized score to overcome the limitations of adapting adult definitions to the pediatric population (53, 57, 58).

Several different methods have also been used to calculate continuous risk factor scores, for instance centile or tertile rankings, principal components analysis, and z-scores (57). In recent years, an increasing number of studies have used standardized single risk factors to calculate clustered risk scores as either the mean or sum of z-scores (57, 59). A z-score is calculated per individual as the amount of standard deviation (SD) from the mean after normalization of the risk factor variable [z = (value – mean)/SD]. After standardization, all risk factors are summed to make the continuous clustered risk score, or the sum can be divided by the total number of included risk variable to denote a mean clustered risk score. The main advantages of using the z-score approach is that the clustered risk score is kept continuous and therefore exploits the full spectrum of available information in data (37). However, a major limitation when calculating z-scores is the use of sample-specific means and SDs. The consequence of is that the calculated clustered risk score also becomes sample-specific, which limits its comparability to risk scores from other populations. So far, the use of sample-specific means and SDs has been standard procedure in the literature (57). Thus, international reference values providing a standard to calculate continuous cardiometabolic risk scores from common means and SDs will make comparison between different study populations possible. Such a unified approach to define levels of cardiometabolic risk in children and adolescents using continuous cardiometabolic risk scores is sorely needed in the field of pediatric epidemiology research and could increase the prospects for estimating and comparing prevalence and trends of cardiometabolic risk in children.

Physical activity

Physical activity is defined as «*any bodily movement produced by skeletal muscles that result in increased energy expenditure*» (60). Dimensions such as intensity (work capacity), frequency (number of sessions per time unit, bouts or days), and duration (units of time) constitute together the total dose or volume of the activity, which yields the energy expenditure associated with PA (61). Physical activity is characterized in a variety of forms (e.g., climbing, bicycling, walking or running) and in different contexts in which the activity takes place (e.g., playground, gym, physical education in school, or transportation) (62).

Valid, reliable and feasible measures of PA are important for drawing meaningful conclusions, for instance, about the prevalence of PA, associations between PA and diverse health outcomes, and the effectiveness of PA interventions (63). However, PA is a highly complex behavior to asses accurately, especially in children. The intermittent and spontaneous nature of children's PA patterns differs considerably from that of adults (64). For instance, most young children spend no more than four continuous minutes at rest, with 95% of activities in high intensity lasting less than 15 seconds (65).

The most widely used methods to assess PA levels in children are by questionnaires (subjective method) and accelerometers (objective method) (61). Questionnaires have traditionally been the preferred approach in large-scale epidemiological studies, due to considerations of budget, study size, and staff availability (66). The use of parent or self-report instruments to measure PA in children can provide valuable insight into the context of PA, but are prone to reporting bias and low validity (67, 68). Technology has made it possible to develop sophisticated PA monitors to overcome these limitations, such as the small wearable accelerometer that provides detailed measures of intensity, frequency, and duration of PA (63). Accelerometers quantify bodily movement by capturing change in velocity over time (m/s^2) in gravity units in one or more planes. Raw acceleration signals are typically not used to express PA, but are converted into accelerometer counts (66). Total PA is often expresses as total counts per minute (cpm), while certain cut-points are used to determine intensity levels (61). Often reported PA intensity levels include low (LPA), moderate-to-vigorous (MVPA), and vigorous (VPA). Sedentary behavior (or time being sedentary) is also frequently reported, characterized by zero to very low accelerometry cpm (63). Sedentary behavior is often defined as "*an energy expenditure ≤ 1.5 metabolic equivalents, while in a sitting, reclining, or lying posture*" (69).

Since the 1980s, when the first accelerometer was developed to capture PA levels (70), accelerometers have become more advanced, lighter and cheaper, with larger memory and battery capacities (70). This has made it far more feasible and accurate for use in large-scale epidemiologic studies. Although several types of accelerometers have been developed, the Actigraph

accelerometer is the most used accelerometer to assess PA and has shown good validity (71-74) and intra-instrumental reliability (74, 75) in children. However, certain methodological considerations need to be addressed when using accelerometers. For instance, thresholds used to identify levels of intensity vary greatly, which complicates direct comparison between studies (63). Other variabilities among data-reduction algorithms used in different studies include the epoch length, non-wear time, criteria's for hours per day that constitute a valid day, and criteria's for days to constitute a valid measurement (63). Other limitations of using the accelerometer include inaccurate measures of some activities, such as incline walking, water-based activities, upper-body movement and cycling. Despite these limitations of the accelerometer, studies have consistently shown that PA levels are higher in boys than girls, that children are the most active members of the population and that PA levels decrease with age (76, 77). Some studies also suggest that patterns of both PA and inactivity track from childhood into adulthood (78, 79).

Physical inactivity is defined as insufficient amounts of MVPA to meet present PA recommendations (69). The latest recommendations for PA for children 5- to 17-years of age from WHO are as follows: 1) to reach a minimum of 60 minutes of MVPA daily, 2) Additional health benefits are provided with higher amounts of daily PA than 60 minutes, 3) At least three times a week, VPA (including activities which strengthen muscle and bone), should be incorporated (80). The Norwegian Directorate of Health has adapted the WHO recommendations for PA and also added recommendations for reduced sedentary time in their latest revision in 2014 (81).

Cardiorespiratory fitness

Cardiorespiratory fitness is often described as "*a set of attributes that people have or achieve*" (60) that are related to an individual's ability to perform PA. More precisely, CRF reflects the ability of the respiratory and circulatory systems to efficiently deliver oxygen to the working muscles under sustained PA (60).

The gold standard to determine CRF is a direct measure of maximal oxygen consumption (VO_{2max}), which expresses the greatest rate at which an individual is able to consume oxygen during sustained and exhaustive exercise (82). VO_{2max} is typically measured during a maximal graded exercise test on a treadmill or cycle ergometer and is expressed as either liters of oxygen consumed per minute (l/min) or as milliliters of oxygen consumed per minute per kg body weight (ml/kg/min). Children do not always reach a VO_2 plateau, and VO_{2peak} has therefore gradually become more frequently used in the pediatric literature to define the highest VO_2 level (83). VO_{2peak} and VO_{2max} have the same

physiological implications as key determinants of the cardiorespiratory system`s functional capacity (84). It has been demonstrated, that children who perform an acceptable VO_{2peak}-test but do not reach a VO_2 plateau, do not have lower VO_2, heart rate (HR) or blood accumulation than those children who do exhibit a plateau (85, 86).

Measures of direct VO_{2peak} are both expensive and time-consuming in large-scale studies, thus variants of indirect maximal exercise tests to measure or predict VO_{2peak} have been developed. The most commonly used indirect fitness tests involve distance/timed runs or shuttle runs, for instance the 20 meter shuttle run test (87) or the Andersen test (88). The 20-meter shuttle run test (MSSRT) has been shown to be a reliable and valid method to estimate VO_{2peak} levels in children (87, 89, 90). The subjects run forth and back for 20 meters, with an initial running pace of 8.5 km/h and with the prerecorded frequency of signals set to increase their speed by 0.5 km/h each minute. The maximal performance is reached when the test person fails to reach the end lines on two consecutive occasions before the set beat (87). The Andersen test is a 10-minute intermittent running test, which also has been shown to be a both reliable and valid measure to estimate VO_{2peak} levels in children (88, 91, 92). As in the MRSST, the children run forwards and back for 20 meters, but in contrast to the MSSRT, the Andersen test is a stop and go test with a 15-second run and a 15-second pause. Thus, no children are excluded during the test which lasts a total of 10 minutes (88). Furthermore, the Andersen test has been shown to be a better indicator of metabolic health than both direct measures of VO_{2peak} and time to exhaustion in children (93).

Since maximal exercise tests are developed to stress the body to exhaustion and can be inadequate in people whose performance may be limited because of pain or fatigue rather than exertion, submaximal tests have also been developed to ease the load on the subjects being tested (94). Submaximal tests can be classified as either performance tests or predictive tests. Performance tests measure responses to standardized PA that typically reflect functional activities in everyday life and are mostly used for adults with musculoskeletal limitations and less frequently used in children (94). Predictive submaximal tests typically uses heart rate (HR) and VO_2 at two or more workloads (95). A VO_{2max} can be predicted by extrapolating the relationship between HR and $\dot{V}O_2$ to age-predicted maximal HR. An example of a submaximal exercise test is the much used Åstrand and Rhyming (Å-R) Cycle Ergometer test, which uses a nomogram in the estimation of VO_{2max} (96). Submaximal exercise tests have in general shown lower reproducibility than maximal exercise tests (97), and concerns have been raised about the margin of error, leading to either over- or underestimation in predicted VO_{2max} values (97-99). For instance, Kasch (98) reported that $\dot{V}O_{2max}$ using the Å-R Cycle Ergometer test was underestimated by 21% in men (aged 30 to 66 years), while Hartung et al. (99) found overestimated $\dot{V}O_{2max}$ values in women (aged 19 to 70 years) by 3 to 21%. In adolescents, a study by

Andersen et al. (97) showed that 82% of directly assessed $\dot{V}O_{2peak}$ in boys and 91% in girls could be predicted using Åstrand's nomogram.

Physical activity, cardiorespiratory fitness and cardiometabolic risk

Although the health benefits of PA were recognized as early as the 5th century BC by the Greek physician Hippocrates, it was not until the landmark work by Jeremiah Noah "Jerry" Morris and colleagues in the early 1950s that this knowledge became scrutinized scientifically (100). By comparing active conductors to sedentary drivers on London's double-decker buses, Morris and colleagues found that occupational PA was inversely associated with CHD (101). This started a new era of PA epidemiology, which for more than half a century has been extensively investigated in the adult population. During the recent decades, children have also become a population group of great interest for the field.

In adults, large, long-term epidemiology studies have provided evidence of a strong inverse association between PA and cardiometabolic risk and overall mortality (100, 102-107) – even independent of genetic factors and many of the traditional cardiometabolic risk markers (100, 108, 109). Regular PA is important for levels of health-related fitness, and both epidemiological studies and clinical evidence have shown that CRF is a strong determinant for overall mortality and morbidity from cardiometabolic diseases (25, 110-115). Furthermore, CRF can in addition to a single or several other traditional risk factors significantly improve the predictive utility of adverse health outcomes (25, 100, 110-113). Importantly, reduced mortality risk has been observed in individuals who improve their CRF (116, 117). Overall, a strong dose-response relationship seems to exist between PA/CRF and clustering of cardiometabolic risk factors, CVD and all-cause mortality in adults (25, 100-115). These dose-response relationships have often been revealed to be curvilinear, with the largest difference being between those individuals exhibiting the lowest level of PA or CRF and the second lowest level (102, 115).

In children, regular PA is associated with a multitude of health benefits and is important for healthy growth and development (48, 78, 118). In addition, PA has been shown to be positively associated with children and adolescent's self-concept, self-confidence, lower anxiety and reduced signs of depression, as well as improved academic performance (118, 119). Although the evidence-based health benefits of PA and CRF on cardiometabolic health in children started to receive more attention in the late 20th century, it has been difficult to provide clear evidence of a precise connection between these. In a review by Caspersen et al. (120) from 1998, the authors stated that

"*…(PA) epidemiologic research applied to children and adolescents is still in its infancy".* The inconsistency and lack of a strong relationship has, to a large degree, been attributed to the large variations in single cardiometabolic risk factors and PA. Traditionally, PA has been self-reported by questionnaires, which as earlier mentioned, introduce subjective errors and low validity. Studies using accelerometers to obtain levels of PA have found more consistent and significant findings, although the associations with single cardiometabolic risk factors often have been weak (37, 121). While measurements of PA express an individual behavior that is highly variable over days, CRF expresses an attribute that is fairly stable over a longer period of time (122). Since the error variations in measures of CRF are less than those for PA (123-125), the association between CRF and single cardiometabolic risk factors in children has been shown to be stronger (126, 127). The association between PA or CRF and cardiometabolic health has later been supported and reinforced in studies showing a consistent inverse association between PA (36, 122, 128-130) and/or CRF (129-133) and clustered cardiometabolic risk. For instance, in the European Youth Heart Study (EYHS), Andersen et al. (36) found a graded inverse association between total PA and clustered cardiometabolic risk, where subjects in the three lowest quartiles of PA exhibited the highest risk. A later study from the EYHS by Anderssen et al. (131) showed that low CRF was a strong predictor of clustering of cardiometabolic risk factors in children. The odds ratios for having clustering in each quartile of CRF were 13.0 [95% confidence interval (CI) 8.8–19.1], 4.8 (95% CI 3.2–7.1), and 2.5 (95% CI 1.6–3.8), respectively, using the highest quartile of CRF as a reference, and after adjusting for country, age, sex, pubertal stage, socio-economic status, family history of diabetes and CVD (131). Thus, observational studies show that a dose-response association between PA/CRF and clustering of cardiometabolic risk factors also seems to exist in children, however, the nature of this response (e.g., linear or curvilinear) is still not fully clear (48). A systematic literature review by Janssen and LeBlanc (48) from 2010 concluded that experimental studies associate higher levels of MVPA and aerobic-based activities (which stress the respiratory and cardiovascular system) with improvements in cardiometabolic health in children and adolescents. However, the review did not apply exclusion criteria based on how PA measures were obtained (e.g., questionnaire, activity diary, pedometer, or accelerometer). A subsequent systematic review by Poitras et al. (134) in 2016 investigated the relationship between objectively measured PA and health indicators in children and found that higher intensities of PA (e.g., MVPA and VPA) showed more consistent and larger effect sizes on cardiometabolic health than activity at lower intensities (e.g., LPA and MPA), however, all levels of PA were found to be important. The findings also indicated that equivalent volumes of sporadic and bouts of MVPA had an equal impact on obesity, CRF, and cardiometabolic risk (134). This has been supported in a recently published study by Tarp et al. (135) of approximately 30,000 children and adolescents, showing that the main determinant of variation in cardiometabolic risk factors was time

spent in higher intensity PA, regardless of the bout duration. Thus, higher intensity PA seems most important for cardiometabolic health, but it appears that no consecutive duration must be reached to achieve health benefits, and PA can therefore be accrued in small doses during the day. The predictive validity of PA and CRF on later CVD mortality and morbidity is not as clear in children as in adults. Studies show that PA may track from childhood to adulthood (136, 137), thus indicating that PA in childhood can have consequences for adverse health outcomes later in life. The Cardiovascular Risk in Young Finns Study has found that PA levels in boys at age 9 and 15 was associated with carotid artery elasticity 21-years later in life, but not with carotid artery intima media thickness, and no associations was found between PA in girls and any of the measures (138). Physical activity is affected by many transitions and life-changing events throughout life and the stability of PA has in general shown to be very low in children (136). Evidence has indicated that CRF in childhood and adolescence is a strong predictor of cardiometabolic risk factors, such as high BP, abnormal blood lipids, and central obesity later in life (139). The predictive validity of CRF for the metabolic syndrome and arterial stiffness, as well as changes in CRF with later cardiometabolic risk factors, has, nevertheless, been shown to be only moderate (139). Overall, the body of pediatric epidemiological literature shows a beneficial association of total PA, higher intensity PA, and higher CRF with a healthier cardiometabolic profile in childhood and adolescence. It has also shown a possible effect of PA or CRF early in life on later cardiometabolic health.

The potential of physical activity to improve cardiometabolic health

Physical activity has the potential to reduce cardiometabolic risk factor levels through several physiological mechanisms (47). These relationships are complex and involve a myriad of interacting functional, metabolic, and morphological adaptations (140, 141), thus only some aspects of the potential effects of PA on the traditional cardiometabolic risk factors and CRF are presented. Exercise refers in the following to a subgroup category within PA often defined as a planned, structured, and repetitive bodily movement, exhibited to improve or maintain one or more components of CRF (e.g., via MVPA) (60).

Muscle fiber mitochondrial volume, aerobic enzyme activity (142), and enhanced capillarization of muscle fibers (143), are positively associated with regular PA and exercise. This improves the metabolic capacity of the muscle, which results in several beneficial effects, such as improved nutrient metabolism. Exercise can improve glucose tolerance by improving both insulin sensitivity and blood glucose control, by enhancing glucose uptake through both insulin- dependent and independent pathways. The beneficial effects of exercise on insulin-dependent glucose uptake are

primarily caused by an increase in GLUT-4 protein expression (144) and in enzymes involved in glucose oxidation and glucose transport-phosphorylation (145, 146). The insulin-independent pathway is stimulated by muscle contractions, which activate a number of signaling proteins of which the adenosine mono-phosphate-activated protein kinase (AMPK) plays a pivotal role for GLUT-4 protein translocation and glucose uptake (147). The AMPK is regulated by the cells' energy status (148) and independent of insulin sensitivity in the muscles (148).

Exercise increases the metabolic rate and fat catabolism (149), and a single bout of exercise can favorably modify blood lipid concentrations by reducing levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG) and elevating HDL-c (150). The lipid enzyme profile can improve with regular exercise by increases in the activities of intravascular enzymes and transfer proteins, such as increased activity of lipoprotein lipase (151) and lecithin-cholesterol acyl transferase (152), as well as decreased activity of cholesterol ester transfer protein (153). These changes may reduce plasma TG and increase HDL-c (154), along with reducing TC and LDL-c (if accompanied by fat loss). It has been proposed that exercise-induced fat oxidation in the cell and reduced intra-myocellular lipid accumulation also decrease insulin resistance (155).

Physical activity can also help maintain healthy body weight and with a sufficient dose and intensity, cause a reduction in body fat in overweight children (156). A decrease in markers of low-grade inflammation, characterized by an excess release of inflammatory markers (including cytokines and hormones) especially from the visceral adipose tissue (157), has been observed with regular exercise in children (158). This has positive implications for cardiometabolic health, since markers of low-grade inflammation have been found important in all stages of the atherosclerotic process (159, 160).

Regular exercise has especially shown beneficial effects on BP in hypertensive (161) and obese (162) children. Although the mechanisms for reductions in BP are not fully understood, these changes may be caused by increased endothelial nitric oxide synthase activity, decreased resting sympathetic activity and adrenal outflow, and insulin sensitivity (163, 164). In addition, loss of fat mass in an overweight child could potentially decrease the vasculature needed to support the adipose tissue and thus, result in lower BP (47).

Cardiorespiratory fitness has not originally been regarded as one of the traditional cardiometabolic risk factors in the metabolic syndrome, but it arguably has a strong relationship to cardiometabolic health. Whereas PA is a behavior, CRF is a physiological trait from habitual PA comparable to other cardiometabolic traits. The hereditability of CRF is >50% (165), but regular exercise of especially vigorous intensity has been shown to contribute to higher levels of health related fitness in children

(118, 166). Exercise can influence CRF due to adaptive responses in the respiratory, circulatory and muscular system. Cardiorespiratory fitness is directly connected to the integrated function of these systems, including pulmonary ventilation and oxygen diffusion, cardiac systolic and diastolic ventricular function, ventricular-arterial coupling, the ability of the circulatory system to efficiently supply and match oxygen requirements in skeletal muscles during exercise, and the muscle cells' ability to receive and utilize the oxygen and nutrients as well as feedback signaling to the cardiovascular center in the brain (medulla oblongata) of these metabolic demands (113). Thus, CRF may be considered as a reflection of total-body health.

The potential effects of PA can therefore be far-reaching for reducing cardiometabolic risk and thus, improving general health. These relationships are more complex in children than in adults due to the influence of growth and maturation, however, the current state of knowledge strongly suggests that regular participation in PA can modify the cardiometabolic risk profile of children (47, 48).

The school as an arena for interventions of physical activity

The school setting is endorsed as an ideal environment to implement population-based interventions of PA (27, 28). First, most children attend school until they reach adolescence, and where a significant part of their waking time is spent. Hence, no other institution influences on children as much during their first two decades of life as the school setting (167). The school may therefore be one of the only arenas for reaching most or all children, ensuring PA in a non-stigmatizing way regardless of age, gender, weight status, fitness level, socioeconomic background, and parental behavior or attitude toward PA. Second, the school can offer a stable schedule and provide permanence of increased habitual activity in children from an early age. Children's PA levels drop significantly at the age of 6 (the time when most children enter school) and continue to drop until adolescence (76). Although the school alone cannot solve the general problem of inactivity, initiatives to increase PA during school time do have the potential to ensure that students meet guidelines for recommended daily PA (168). Thus, the school can be an important arena for interventions of PA from a public-health perspective.

Several school-based intervention studies have been conducted during the two last decades with various degrees of success to increase PA levels and positively influence cardiometabolic risk factors (including CRF) (169, 170). Overall common features in successful school-based PA interventions, identified in two large literature reviews and a review of reviews (169, 170), include interventions delivered in the school setting by school- or PE-teachers, interventions that are a mandatory part of

the school curriculum (rather than voluntary) and multicomponent interventions (e.g., PA/PE interventions also including activity breaks and/or family involvement). Implementation research has also provided useful insight into how early engagement of partners and stakeholders are important for a successful implementation of interventions within the school system (171), as well as how anchoring and support within the school system likely increases sustainability (172). Specific factors that impede or facilitate implementation and execution of PA/PE interventions within the school have primarily been reported to be either institutional- or teacher-related (173, 174). Institutional factors include the (teachers') perceived priority of the intervention in the school, support from school boards and school administrators, scarce resources, lack of facilities or equipment, and/or a crowded curriculum. Among the most frequently reported teacher-related factors are low levels of confidence in conducting the physical activities or teaching PE, lack of knowledge, and level of expertise and/or qualifications (173, 174). School-based interventions aim to implement research in a real-world setting. Thus, comprehensive considerations from researchers should take into account the busy school day for students, teachers and principals. Since the majority of school-based PA interventions involve teachers as facilitators or primary delivery agents (169, 170, 175), it is essential that teachers should be provided with sufficient information, tools, support, and education to empower and qualify them to deliver the intervention.

In 2009, the Norwegian government decided to introduce a new school subject "physical activity and health" (PAH) for 5th- to 7th-grade schoolchildren, adding in total 76 additional hours of PA to the curriculum over three school-years. The aims of PAH were to increase PA levels and provide more variety in the school day for students. Eight years later, the Norwegian Parliament (2017) decided to prepare a work program for an initiative of 60 minutes of PA for all schoolchildren between 1st and 10th grade. Concerns about reducing time in other subjects led to the idea of integrating PA in academic lessons and make use of short-term breaks, inspired by school-based PA studies such as the Norwegian ASK study (176). The ASK study was a school-based randomized-controlled trial conducted in the school year 2014-2015 in Western Norway. The evaluation of the effects of the ASK study on cardiometabolic risk factors is a part of this present thesis.

Research aims

A common standard to define cardiometabolic risk in pediatric populations when using continuous clustered risk scores is highly warranted in the epidemiological literature. Such reference values could aid comparability between studies and increase the ability to estimate and compare prevalence rates and trends of cardiometabolic risk in children and adolescents. There is a further need to evaluate the effects of initiatives to increase PA in schools on cardiometabolic risk, which also takes the limitations and barriers identified in earlier school based studies into consideration and which can provide a sustainable and feasible school-based PA intervention model. Such initiatives could give a mandate for the continuing interest of the Norwegian government in investing in increased PA in the Norwegian school system. The aims of this thesis were threefold:

First aim

To provide international age- and sex-specific reference values for cardiometabolic risk factors in children and adolescents (**paper I**).

Second aim

To investigate cardiometabolic risk factor levels in Norwegian children from the ASK study compared to international standards, and examine the association between CRF and the reference-standardized clustered risk score (**paper II**).

Third aim

To investigate the effects of the ASK cluster-randomized school-based PA intervention on single and clustered cardiometabolic risk factors in Norwegian children (**paper III**).

Materials and methods

Reference values for cardiometabolic risk scores

Sample and study design

Data from 23 cohorts of children aged 6–18 years were pooled (58). Data were divided into eight new cohorts for this study, representing data from the following countries: Denmark (EYHS 1997–1998 and 2003–2004) and Copenhagen School Child Intervention Study (CoSCIS 2001, 2004, and 2008)), Estonia (EYHS 1999), Norway ((EYHS 1999–2000) and Physical Activity among Norwegian Children Study (PANCS) 2005–2006), Portugal (EYHS 1999–2000 and 2008), Switzerland (Kinder Sports Studie (KISS) 2005–2006 and 2009), and the United States (US National Health and Nutrition Examination Survey (NHANES) 2001–2002, 2003–2004, 2005–2006 and 2007–2008).

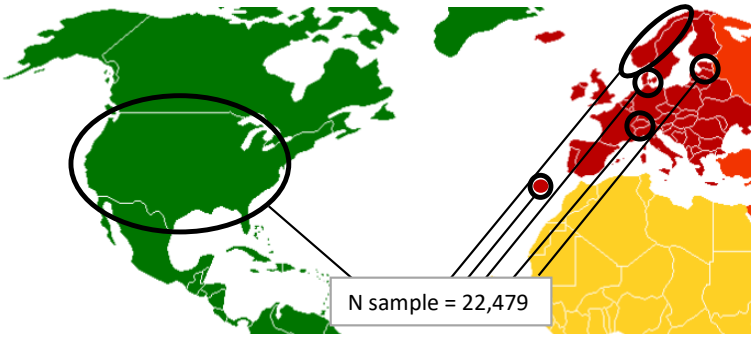

Figure 2. North American and European countries contributing with data to the pooled database.

The majority of the European data (EYHS, KISS, and PANCS) were based on randomly selected samples, using public schools as the primary sampling unit. In PANCS, Statistics Norway randomly selected schools from all regions of the country that included 96% of all Norwegian children in the 4th and 10th grades in the sampling frame. Of those, 82% (n = 2,299) participated and n = 2,266 were included in the present study (177). In EYHS, a two-stage cluster sampling procedure was used to recruit a minimum of 1,000 girls and boys (9 and 15 years old) from each study location, randomly sampled from the schools' register lists. Schools were stratified according to the socio-economic character of the local area (urban or rural) and weighted according to size (178). The overall participation rate was 74% in EYHS I (n = 4,169), whereof this study included children from Estonia (n = 1,174) and Norway (n = 754) (178). We also included data from Danish (n = 1,861) (179) and Portuguese (n = 1,771) children from both EYHS I and EYHS II. The KISS PA intervention study represents data from two provinces of Switzerland, where 28 classes were randomly chosen from a sample of 190 consenting classes. Of the initial 502 children included at baseline (age 7 and 11 years), 96% were re-tested at the post-intervention tests (180) and 60% at the 3-year follow-up (181). We

excluded follow-up values from KISS for variables where a significant intervention effect was revealed. In total, 1,309 observations were included from the KISS study in the present study. The cohort in CoSCIS PA intervention study consisted of representative Danish children from two suburbs of Copenhagen. In total, 696 children (68% of those invited) participated at baseline from 46 preschool classes (aged 6–7 years), 613 of these were included in the post-intervention tests, and 513 in the 7-year follow-up (182). We included all values from CoSCIS as no intervention effects were revealed between groups (n = 1,812 observations). NHANES randomly selected representative North American children following a multi-stage selection procedure, using mostly single counties as the primary sample unit with probability proportionate to the measure of county size (183). Compared to Andersen et al. (58), we extended the NHANES data to cover the same time period as that covered by the European data and to match the numbers of European and American children. Overall, n = 11,532 children from the NHANES were included in the present study from biannual survey releases covering 7 years (2001–2008). In total, 22,479 valid observations (48.7% European vs. 51.3% American), 11,234 from girls and 11,245 from boys, in at least one of the 14 cardiometabolic risk variables of interest, were included. Please see **Table 1**.

All individual studies have been ethically approved prior to commencement of investigation and all participants and legal guardians provided informed consent. Details of the earlier pooling of cohorts, sampling procedures, data collections, analyses, and ethical approvals for the separate studies are described elsewhere (58, 177, 178, 182-185).

Cardiometabolic risk factors

The variables included were systolic blood pressure (SBP), diastolic blood pressure (DBP), waist circumference (WC), body mass index (BMI), sum of four skinfolds (Sum4Skin), TG, TC, HDL-c, LDL-c, the ratio of TC to HDL-c (TC:HDL-c ratio), glucose, insulin, homeostatic model assessment (HOMA) score and CRF.

Anthropometry

WC was measured with an anthropometric tape around the abdomen at the end of a light respiration, either a) at the level of the umbilicus, b) midway between the lower rib margin and the iliac crest, or c) the natural waist (at the smallest circumference between the ribcage and the iliac crest and half-way between the lower costal rib and the Spina iliaca anterior superior). WC was measured once in all studies except EYHS, which obtained two measures of WC and the mean values of those was used in the analysis.

Table 1. Cohorts and cardiometabolic risk variables represented in the reference material

Cohorts	Year	N	Age range (years)	SBP	DBP	WC	BMI	S4S	TC	HDL	LDL	TC:HDL	TG	Glucose	Insulin	HOMA	CRF
Denmark (EYHS)	1997 & 2003	1861	8-11 15-18	x	x	x	x	x	X	x	x	x	x	x	x	x	x
Denmark (CoSCIS)	2001, 2004 & 2008	1812	6-14	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Estonia (EYHS)	1999	1174	8-11 14-18	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Norway (EYHS)	1999	754	9-10 15-17	x	x	x	x	x	x	x	x	x	x	-	-	-	x
Norway (PANCS)	2005 & 2009	2266	9-11 15-17	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Portugal (EYHS)	1999 & 2008	1771	8-10 15-18	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Switzerland (KISS)	2005,2006 & 2009	1309	6-17	x	x	x	x	x	x	x	x	x	x	x	x	x	x
USA (NHANES)*	2001-2008	11532	6-18	x	x	x	x	-	x	x	x	x	x	x	x	x	x

Abbreviations: BMI: body mass index; CoSCIS; Copenhagen School Child Study, CRF: cardiorespiratory fitness; EYHS; European Youth Heart Study, HDL: high-density lipoprotein; HOMA: homeostatic model assessment; KISS; Kinder Sports Studie; LDL: low-density lipoprotein; MAP: mean arterial pressure [DBP+ (SBP-DBP/3)]; N: number of observations; NHANES; National Health and Nutrition Examination Survey, PANCS; Physical Activity among Norwegian Children Study, SD: standard deviation; S4S: sum of four skinfolds; TC: total cholesterol; TC:HDL: total cholesterol: high-density lipoprotein; TG: triglyceride; WC: waist circumference. * NHANES did only obtain measures of glucose, insulin, and CRF for children aged≥ 12 years. N total, 22 479 observations (n girls, 11 234; n boys, 11 245) with valid values in *at least* one of the 14 cardiometabolic risk variables.

Height was measured without shoes to the nearest ≤ 0.5 cm, and body weight was measured with an accuracy of ≤ 0.5 kg. BMI (kg/m^2) was calculated as weight (kg) divided by height squared (m^2). Skinfold thickness was measured with a Harpenden caliper at biceps, triceps, subscapular, and suprailiac sites and summed for the use in analyses.

Blood pressure

Similar for the studies included, SBP and DBP were measured when children were in a sitting position after at least 5 minutes of rest, at the mid-upper arm with an appropriately sized cuff. The European studies all used oscillometric blood pressure devices, whereas NHANES used the auscultation method with a mercury sphygmonometer. The mean of three measures was used in the analyses (if more than three measures were obtained, the mean of the last three was used). s

Blood samples

Blood samples were collected during the morning hours in fasting children and stored at -70 to -80°C until analysis. All studies drew intravenous blood samples from participants except the Norwegian part of the EYHS, which used capillary blood as the basis for the biochemical analyses. Children under the age of 12 years were not instructed to fast in NHANES, and non-fasting TC, HDL-c, and TG values were therefore corrected for fasting time (hours) according to Steiner et al. (186). TC, HDL-c, and TG were measured by enzymatic methods, and LDL-c estimated from TC, HDL-c and TG using the Friedewald formula (187). Glucose was analyzed using the hexokinase method in all studies except CoSCIS, which used the dehydrogenase methodology. Insulin was measured using an enzyme-linked immunosorbent assay. No values for glucose or insulin were obtained for children < 12 years in NHANES. HOMA score was defined as [insulin (pmol/L) * glucose (mmol/L)]/135 (188).

Cardiorespiratory fitness

Cardiorespiratory fitness (mL/kg/min) was assessed by: a) directly measured VO_{2peak} from a graded maximal treadmill running test (189), b) directly measured VO_{2peak} during a graded maximal cycle ergometer test (177), c) a standardized submaximal treadmill running test (190), or d) the 20-meter graded maximal MSSRT (87). All results obtained from cycle ergometer tests were multiplied by 1.05 to be comparable to results obtained by the directly measured VO_{2peak} treadmill running test (191). NHANES did not measure CRF in children < 12 years. More detailed test descriptions, are described in the original studies (58, 177, 178, 182, 184, 185).

The Active Smarter Kids Study

Sample and study design

The ASK study was a seven-month cluster-randomized controlled trial conducted November 2014 to June 2015 in Sogn and Fjordane county, Western Norway (176). The inclusion criteria were classes including at least seven students, that the individual child should the able to participate in both regular daily PA, PE and being able to complete academic performance tests. Sixty invited schools, encompassing 1,202 fifth-grade children, were randomized on a 1:1 ratio by a neutral third party (Centre of Clinical Research, Haukeland University Hospital, Norway). Following randomization, three schools (two intervention schools, n children = 16, and one control school, n children = 11), withdrew from participation.

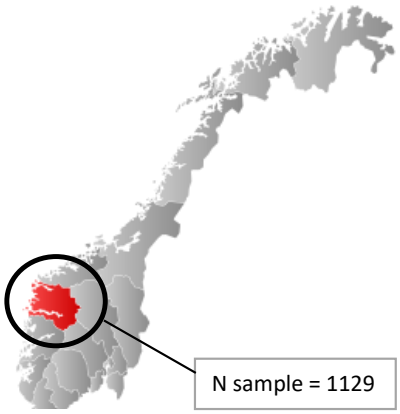

Figure 2. Sogn and Fjordane county, Western Norway.

In total, 1,145 out of 1,175 invited children agreed to participate (82% of the 10-year-old population in the county). Seven of the children dropped out during the intervention period (see flowchart in Figure 3). In total, valid data was obtained from 1,129 fifth-graders from 57 schools.

Baseline measures of PA were obtained from April to June 2014, while cardiometabolic risk factors (including CRF) were measured from August to October 2014. Follow-up measures were conducted simultaneously from April to June 2015. In addition, PA levels were measured mid-way through the intervention period.

Intervention

The PA intervention consisted of three components, engendering 165 min of additional PA per week for the intervention children compared to the control children: 1) physically active academic lessons executed in the playground (3 x 30 min per week), 2) PA breaks during classroom lessons (5 min per school day), and 3) PA homework prepared by the teachers (10 min per school day). The intervention was a mandatory part of the school curriculum in intervention schools and was added to the amount of existing mandatory curriculum-prescribed PA (45 min per week) and PE (90 min per

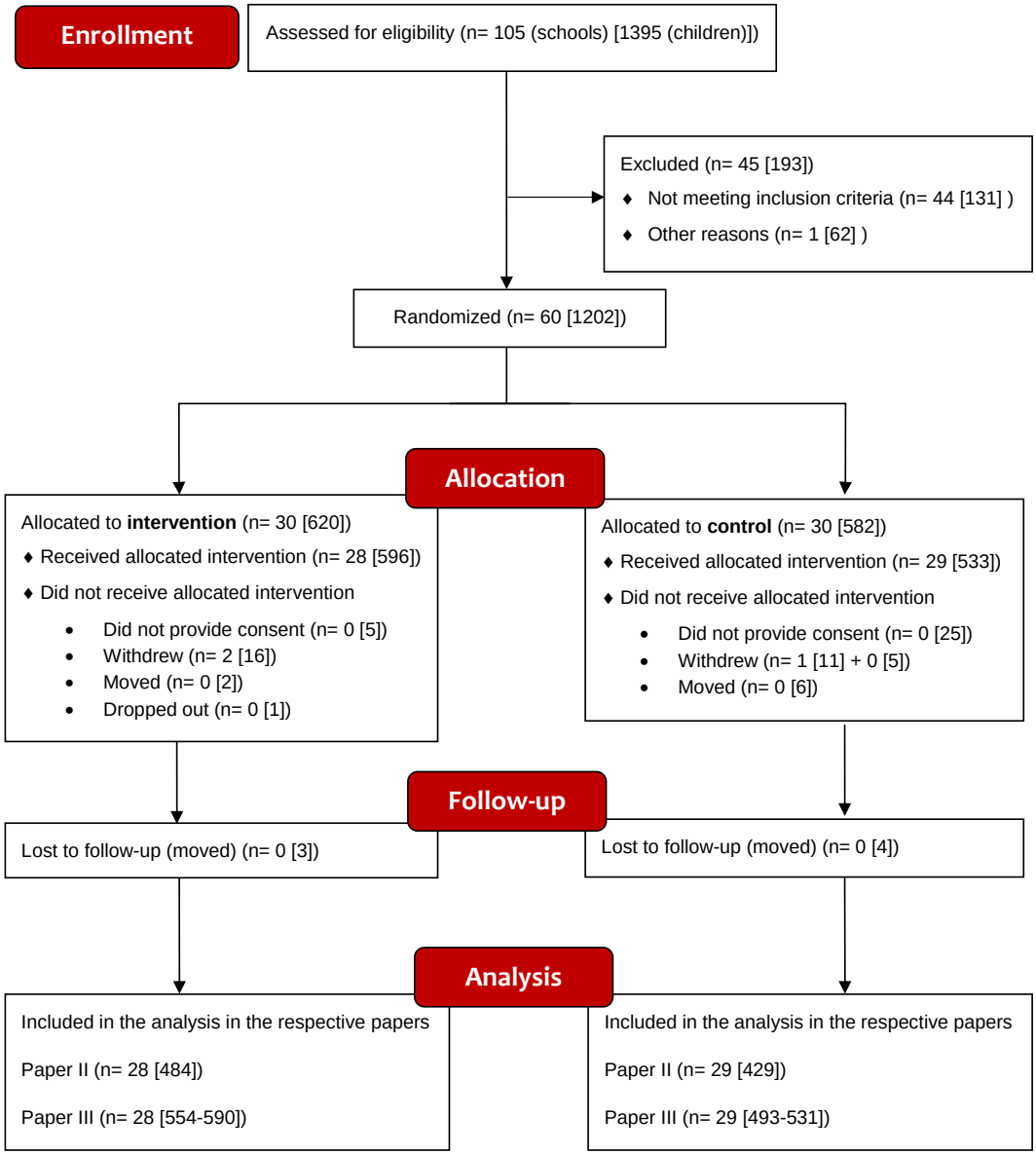

Figure 3. Consort flowchart of the enrollment, allocation, and follow-up of ASK-children and final included population in the statistical analysis.

week), which totaled approximately 135 min per week. In total, the intervention school children were exposed to 300 min PA/PE per week. Control schools were encouraged to provide only the mandatory amount of PA/PE (135 min per week). The intervention was delivered by the fifth-grade

classroom teachers. Three comprehensive pre-intervention seminars over five months and two regional refresher meetings during the intervention period were conducted to empower, support and qualify the teachers to deliver the intervention. These meetings were also meant for intervention school teachers to share experiences and solve challenges with each other and the research team. In addition, we provided teachers from intervention schools with email- and telephone-support. A password-protected website (http://www.askbasen.no/), accessible only to intervention school teachers during the intervention period, made it easy for the teachers to be inspired by videos and examples of physically active lessons in their everyday settings. We aimed to provide activities that were inclusive, joyful, and varied so that unfit or unenthusiastic children also could feel motivated to participate, and teachers were encouraged to provide a positive and mastery-oriented learning environment to increase children's sense of self-efficacy and positivism toward the activities. Approximately 25% of the daily PA in school was intended to be of vigorous intensity; the children should be "*sweating and out of breath*". All Intervention schools received a large "tool-box" with equipment (e.g., mathematics dice and bingo tiles, cones and laminating machines etc.) to perform the intervention. The children in the intervention schools received a basic package including a skipping rope and a ball for their PA homework.

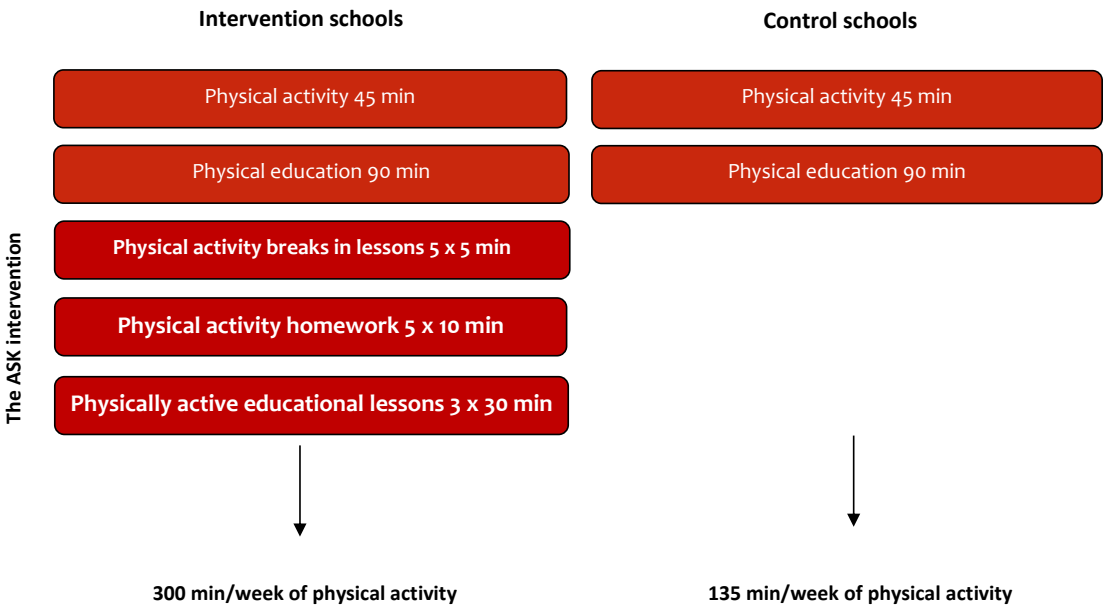

Figure 4. The physical activity intervention in the ASK study

Physical activity

Physical activity was measured using the ActiGraph GT3X+ accelerometer (ActiGraph GT3X+, LLC, Pensacola, Florida, USA). Children were instructed to wear the accelerometer at the right hip at all times over seven consecutive days, except during water-based activities/showering or while sleeping. Valid monitor wear-time was defined as reaching ≥480 min per day between the hours of 06:00 and 24:00 and a valid school-day as reaching ≥180 min per day between 09:00 and 14:00. Periods of ≥20 min of zero counts were defined as non-wear time (192). Children having valid wear-time in ≥4 (out of 7) days and in ≥3 (out of 5) school days were included in the analysis. Previously established and validated cut points were used to define sedentary time (<100 counts per minute (cpm)), MVPA (>2296 cpm) and VPA (>4012 cpm) (193, 194). KineSoft analytical software (KineSoft version 3.3.80, Loughborough, UK) was used to analyze all accelerometer data, using data accumulating at ≥ 10 sec epochs. Accelerometer data was collected at baseline (April-June 2014), mid-way through the intervention (January-February 2015) and at follow-up (April-June 2015).

Adherence to the physical activity protocol

On a monthly basis, intervention school teachers reported the weekly amount of PA (duration in minutes and intensity), while control schools reported once every semester. Intervention school teachers reported for all three components of the intervention (PA educational lessons, PA breaks, and PA homework). Intensity of the PA was defined as 1 = low intensity, 2 = moderate intensity, and 3 = vigorous intensity. All 57 schools also reported absences from school for each child.

Anthropometry

BMI

Body mass was measured to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany). Children were wearing light clothing, though underwear was preferred based on the acceptance of the child. A portable Seca 217 (SECA GmbH, Hamburg, Germany) was used to measure stature to the nearest 0.1 cm with the barefooted child facing forward. BMI ($kg*m^{-2}$) was calculated as weight (kg) divided by the height squared (m^2).

Waist circumference

Waist circumference was measured using an ergonomic circumference measuring tape, Seca 201 (SECA GmbH, Hamburg, Germany). Two measures were taken between the lowest rib and the iliac crest to the nearest 0.5 cm, with the child's abdomen relaxed at the end of a gentle expiration. If the measures differed >1 cm, we obtained a new measurement. The mean of the two closest measurements (≤ 1 cm) was used for analyses.

Blood pressure

Systolic BP and DBP were measured by the Omron HEM-907 automated BP monitor (Omron Healthcare, Inc, Vernon Hills, IL, US). The device is validated according to the AAMI validation protocol (195) and to the validation criteria of the International protocol for blood pressure measuring devices (196). The children were measured in a quiet room after resting for 10 minutes in a sitting position (without distractions) on the upper right arm. Four measures were taken with 1-minute pauses in-between, and the mean of the last three measures was used for analysis. If the measures differed more than 5 mmHg, a new measure was obtained and the mean of the last four blood BP measures was used.

Blood samples

A nurse or phlebotomist collected intravenous blood samples from the children's antecubital vein after an overnight fast (lasting from 20:00 to 10:00). A standardized protocol was used to obtain serum, consisting of the following five steps: 1) Blood plasma was collected in 5-ml tubes with gel (Vakuette® Serum Gel with activator, G456073). 2) Tubes were carefully turned upside-down five times and placed vertically for coagulation. 3) The sample was centrifuged after 30 minutes at 2000 G for 10 minutes. Serum was visually inspected for residues and centrifugation was repeated if residue was present. 4) The serum tube was kept in refrigerator at 4 °C before 0.5 ml was pipetted into cryo tubes. 5) Prior to biochemistry analyses, the cryo tubes were stored at -80 °C. Blood serum from both baseline and follow-up were analyzed in one batch by an ISO-certificated laboratory for traditional risk factors related to cardiometabolic diseases, such as insulin, glucose, TG, HDL-c, LDL-c, and TC. A TC:HDL-c ratio was calculated to represent dyslipidemia. Insulin resistance was defined by the homeostatic model assessment (HOMA)-score determined by [insulin (pmol/L) * glucose (mmol/L)]/135 (188).

Cardiorespiratory fitness

Cardiorespiratory fitness was measured with the validated Andersen shuttle-run test (88, 91), following standard procedures. The tests were completed indoors on a wooden or rubber floor in groups of 10-20 children. Children ran a 20-meter distance between two lines in an intermittent pattern, touching the floor with one hand behind the line at each turn. They ran for 15 seconds and stood still for another 15 seconds continued for a total of 10 minutes. The total distance (meters) covered was used as the outcome for analyses and was recorded by adult test assistants. All schools and teachers were provided with a video demonstration of the Andersen test, which they were asked to show the children before the testing. Further, the test was explained and demonstrated by the

test-leader prior to the test, and children performed a 5-minute warm up using the principles from the Andersen test to become familiar with the test procedure.

The performance in the Andersen test was converted into VO_{2peak} by the following equation: boys VO_{2peak} = 27.1689 + (0.0397 × distance (m)) – (0.1698 × body mass (kg)), and girls VO_{2peak} = 32.5793 + (0.0309 × distance (m)) – (0.2351 × body mass (kg)) (197).

Sample size and power calculation

The ASK study was designed to detect an effect size (Cohen`s D) of 0.35 between groups for change in the *main outcome* variable, academic performance. Sample size calculations were performed using standardized formulas, corrected for the cluster-randomized controlled trial design. A sample size of 468 children in both arms (intervention and control) was required, calculated from two steps; First, a naive n = 103 children in each group were found by using standard formulas, given $\alpha = 0.05$, $1-\beta = 90\%$, a group ratio 1:1, and a correlation of repeated measurements = 0.7. Second, the naive n was corrected by the cluster-randomized controlled trial design (design effect = [1 +[(CV2 + 1)*n – 1]* (ICC)] = 4.54), where ICC (the intra-class correlation coefficient) = 0.15 based on observations of clustering of academic performance during the previous school year (2013-2014), n = number in each cluster after accounting for a maximal expected attrition rate of 20%, n = 16.2, and CV (coefficients of variance for n) = 0.72.

The sample size (N = 468 children in both arms) allowed the ASK study to detect a significant difference between groups for variables reaching an effect size of 0.24 (ICC = 0.05) to 0.30 (ICC = 0.10) or higher for *secondary outcomes*, such as cardiometabolic risk variables, accepting the former assumptions (176).

Blinding

Due to the nature of the experiment, blinding of children and schools was not possible. However, only the project management group had formal knowledge of group assignment. The rest of the research group, test assistants, data manager and statisticians were blinded to group allocation until after the follow-up round was finished.

Ethics

The study protocol was approved by the Regional Committee for Medical Research Ethics and all procedures and methods conform with the ethical guidelines of the World Medical Association's Declaration of Helsinki and its subsequent revisions (198). Written informed consent from parents or

legal guardians was obtained prior to commencement of the investigation. The ASK study is registered in the Clinicaltrials.gov registry [NCT02132494].

Statistical analysis

All analyses were conducted using IBM SPSS version 23-25 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). The primary statistical models used in the papers were linear mixed models, including a random intercept to account for clustering of observations within samples. The random intercept of cohort was used in **paper I**, analyzing pooled data from several studies in children, while the random intercept of school was used in **paper II** and **paper III**, analyzing data from the ASK study. Values exceeding five SDs from the mean were classified as (extreme) outliers and excluded from the data material before analyses in all papers.

Reference values for cardiometabolic risk scores

In **paper I**, cardiometabolic reference values were found by testing the trend for age in all linear mixed models by evaluating first-, second-, and third-order terms of age (higher order terms were omitted to avoid overfitting). The highest order terms were retained in the models if statistically significant. Thus, reference values for variables where a linear trend for age was evident were calculated by the following regression equation: risk $factor_{ref\_linear} = \alpha + (\beta * age)$. Reference values for variables showing a quadratic or cubic relationship with age were calculated by including a second-order term (risk $factor_{ref\_quadratic} = \alpha + (\beta_1 * age) + (\beta_2 * age^2)$) or including a second- and third-order term (risk $factor_{ref\_cubic} = \alpha + (\beta_1 * age) + (\beta_2 * age^2) + (\beta_3 * age^3)$), respectively. All analyses were conducted separately in boys and girls after verifying statistically significant interactions by gender (age*gender) for some variables. Age- and gender-specific reference values are presented as means and SD, where SDs were calculated as the mean of residuals from the regression models. All variables and regression equations are presented by gender and age as absolute values. Non-normally distributed variables (WC, BMI, Sum4Skin, TG, TC:HDL-c ratio, insulin and HOMA score) were logarithmically transformed (natural log) before analysis and presented as log-transformed values.

In addition, a subgroup analysis was performed using a broad selection of previously applied continuous clustered risk scores in the pediatric population (57, 59, 133). The analysis was conducted to investigate the comparability of differently constructed clustered risk scores, using the Pearson correlation coefficients. Single risk variables were converted to z-scores, using log-transformed values for those skewed variables, summarized and divided by the total number of included variables to create comparable mean values for the clustered z-scores. Calculations were derived from the

reference values suggested in **paper I**, representing children with valid data in all 14 risk variables (n = 6,471).

The Active Smarter Kids study

In **paper II** and **paper III**, descriptive statistics of the ASK population are presented as means and SD, median and interquartile range (IQR), or numbers and percentage (%).

In **paper II**, skewed variables were logarithmically transformed before analyses by the natural logarithm (Ln): BMI, WC, TG, TC:HDL-c ratio, insulin and HOMA. A linear mixed model and a generalized estimating equation model including school as a random effect were used to examine baseline differences between sexes for the continuous and categorical variables, respectively. To enable comparison of single and clustered cardiometabolic risk factor values between the Norwegian children and the international reference values, we standardized the following risk factors according to the reference values presented in **paper I**; BMI (ln), WC (ln), SBP, DBP, LDL-c, HDL-c, TC:HDL-c ratio (ln), TG (ln), glucose, insulin (ln), HOMA score (ln), and CRF (VO_{2peak}). Each single risk variable was standardized by sex using the following equation; reference-standardized variable (z-score) = $(x+\bar{x})/SD(\bar{x})$, where age-predicted reference values were used as the mean ($\bar{x}$) calculated from regression equations for the single cardiometabolic risk factors (199). A mean clustered reference-standardized risk score of traditional risk factors was calculated by summing up the z-scores WC, SBP, TC:HDL-c ratio, TG, and HOMA score and dividing by five. A second mean clustered risk score was calculated including CRF (VO_{2peak} inversed) by summing up the same reference-standardized risk factors as described above and CRF and dividing by six.

In addition, **paper II** explored the association between the Andersen test and the reference-standardized mean cluster score (without CRF) using a linear mixed model; CRF as the independent variable, the reference-standardized mean cluster score as the dependent variable, and school as a random effect to account for the cluster effect. Age, sex, pubertal stage and SES were included as covariates, but only sex and pubertal stage changed the estimates and were therefore included in an adjusted model. In line with previous studies investigating the association between CRF and clustered cardiometabolic risk factors (93, 122), we investigating whether sex moderated the association between CRF and clustered cardiometabolic risk factors. To produce interpretable beta coefficients, both CRF and the clustered risk score were standardized before analysis. An alpha-level of $p \leq 0.05$ was considered statistically significant for all abovementioned analysis.

Paper III performed effect analyses. First, a continuous clustered risk score was calculated from the age-standardized z-scores WC, SBP, TC:HDL-c ratio, TG, HOMA-score, and CRF (inversed) divided by 6. The residuals of change between baseline and follow-up for the cardiometabolic risk factors were

normally distributed, although some of the single cardiometabolic risk factors were skewed, and the respective risk variables were therefore not transformed.

Three analyses were used to evaluate the effect of the intervention, all conducted according to the statistical analysis plan (176) and by using linear mixed models with school as a random effect to account for the cluster effect: 1) The effect of the intervention was investigated using intention-to-treat analysis, including all children who had valid data on the single cardiometabolic risk factors at both baseline and follow-up. Multiple imputations were also conducted for missing data, imputed from relevant variables using a Markov Chain Monte Carlo procedure. We assumed data were missing at random (200). The models included group and baseline values as independent variables and change as the dependent variable. 2) Per-protocol analysis was conducted using similar models but included data only from intervention schools reporting ≥ 80% of prescribed PA and control schools reporting < 120% of the curriculum-prescribed PA (135 min/week). 3) In line with similar studies (201, 202), we tested the moderating effects of cardiometabolic baseline values and sex. We hypothesized that intervention effects would be larger in girls, as they typically have lower levels of MVPA (76) and CRF (203). The subgroup analyses were performed on children having valid measures in all cardiometabolic risk variables (n= 769), by including the following interaction terms in three separate analyses: a) group*tertile (tertiles were defined by baseline values in the clustered cardiometabolic risk score), b) group*sex, and c) group*sex*tertile. All models were full factorial models: two-way interaction models included main effects, and three-way interaction models included main effects and two-way interactions. The interaction effect by group*tertile (model a) and group*sex*tertile (model c) was investigated in linear mixed models using group, subgroup(s), and the interaction term as independent variables and change as the dependent variable. The moderating effect of group*sex (model b) also included baseline values of the single cardiometabolic risk factor as an independent variable in the respective models.

In **paper III**, analyses were performed using actual units and using z-scores to allow for a meaningful interpretation of results. A p-value < 0.05 was considered statistically significant in analysis of the main effects, whereas a p-value < 0.1 was applied to indicate statistical significance of interaction terms (204).

Summary of results

Paper I

Reference values for cardiometabolic risk scores in children and adolescents – suggesting a common standard

In total, 22,479 observations (48.7% European vs. 51.3% American), 11,234 from girls and 11,245 from boys, aged 6–18 years were included in the study (see **Table 1**). Reference values for 14 of the most commonly used cardiometabolic risk variables in clustered risk scores were calculated and presented by age and gender: SBP, DBP, WC, BMI, Sum4Skin, TG, TC, HDL-c, LDL-c, TC:HDL-c ratio, glucose, insulin, HOMA score, and CRF (**Table 2**). Binary correlation coefficients between 16 different clustered cardiometabolic risk scores, based on the derived reference values, showed an overall high correlation (r = 0.85) (**Table 3**).

Table 2. Regression equations by age for single cardiometabolic risk factors in girls and boys

Equations for actual units are shown for all included variables, whereas equations for log-transformed values are shown only for skewed variables.

	Girls						Boys					
	γ-intercept	β-coefficient			SD	ICC	γ-intercept	β-coefficient			SD	ICC[a]
		age	*age²*	*age³*				*age*	*age²*	*age³*		
					Absolute values							
SBP	84.085419	1.352664	0.100390	−0.005781	8.6722095	0.09	84.930208	1.810944			9.3180619	0.08
DBP	48.251195	1.642089	−0.036912		7.7788888	0.24	54.131674	0.652442			8.0580317	0.26
WC	6.923239	9.461851	−0.494561	0.010098	10.1742481	0.14	30.320353	3.876927	−0.065298		10.6005333	0.06
BMI	7.843299	1.219271	−0.021625		4.1473040	0.06	11.196837	0.650082			3.9862129	0.05
Sum4Skin	18.512255	2.029718			18.3129132	0.10	−0.308554	5.330204	−0.196941		16.7136902	0.06
TC	2.327413	0.613717	−0.057694	0.001662	0.7496077	0.07	4.641488	−0.042366			0.7383655	0.05
HDL-C	0.730070	0.230285	−0.021182	0.000608	0.3196003	0.07	−0.212351	0.504949	−0.044087	0.001157	0.3210060	0.06
LDL-C	2.219779	0.148092	−0.018060	0.000569	0.6603341	0.08	2.006579	0.200585	−0.023807	0.000745	0.6446768	0.08
TC:HDL-C	3.044685	−0.006873			0.7540697	0.12	2.993067	−0.047512	0.003353		0.8018310	0.11
TG	0.407089	0.059863	−0.002143		0.3723611	0.09	0.547833	0.017777			0.4018758	0.07
Glucose	1.493865	0.742463	−0.050012	0.001072	0.3981106	0.16	2.171775	0.588765	−0.037923	0.000797	0.4133370	0.24
Insulin	84.837643	−25.575818	3.268078	−0.109149	30.7104507	0.09	50.370236	−14.543330	2.041363	−0.068083	32.0871352	0.06
HOMA	2.425747	−0.802842	0.112573	−0.003893	1.2376530	0.07	1.861164	−0.569878	0.081108	−0.002707	1.3600340	0.05
CRF	20.809510	5.889239	−0.489963	0.012177	6.6184862	0.19	43.455681	0.441795			7.7436990	0.13
					Natural log-transformed values							
WC	3.340392	0.126343	−0.005788	0.0000974	0.1323175	0.15	3.609076	0.065647	−0.001350		0.1359390	0.07
BMI	2.474502	0.028888	0.002023	−0.000096	0.1801600	0.06	2.544058	0.031888			0.1749271	0.05
Sum4Skin	3.054577	0.050292			0.3769964	0.13	2.502077	0.141056	−0.005106		0.3981823	0.08
TC:HDL-C	1.085626	−0.002610			0.2351238	0.14	1.075531	−0.016854	0.001108		0.2462190	0.11
TG	−0.782962	0.069081	−0.002402		0.4154836	0.11	−0.643920	0.021832			0.4436847	0.08
Insulin	3.021447	−0.178175	0.039234	−0.001501	0.5043443	0.09	2.925177	−0.161127	0.034727	−0.001283	0.5538751	0.10
HOMA	−1.257847	0.033936	0.024693	−0.001179	0.5344390	0.07	−1.210175	0.018537	0.022704	−0.001018	0.5862900	0.07

β: beta; BMI: body mass index; CRF: cardiorespiratory fitness; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment; ICC: intra-class coefficient; log: logarithmically; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; SD: standard deviation; Sum4Skin: sum of four skinfolds; TC: total cholesterol; TC:HDL-C: total cholesterol:high-density lipoprotein cholesterol ratio; TG: triglyceride; WC: waist circumference; γ = regression.

[a] ICC's represents the cluster effect among the cohorts. All models significant, $p \leq 0.001$.

Table 3. Matrix of bivariate correlations of continuous cardiometabolic cluster score approaches used in pediatric populations

		Mean (SD)	Correlations[b]														
Cluster scores	**Girls**		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	DBP + TC + HDL-C	0.04 (0.51)	1															
2	Average (SBP + DBP) + HDL-C + TG + Glucose + WC	−0.03 (0.50)	0.63	1														
3	Average (SBP + DBP) + HDL-C + TG + HOMA + S4S	−0.01 (0.56)	0.60	0.88	1													
4	Average (SBP + DBP) + HDL-C + TG + Glucose + Insulin + WC	−0.03 (0.50)	0.57	0.96	0.93	1												
5	MAP + HDL-C + TG + WC	−0.05 (0.53)	0.70	0.91	0.89	0.86	1											
6	MAP + HDL-C + TG + HOMA + BMI	−0.03 (0.53)	0.62	0.89	0.96	0.95	0.91	1										
7	MAP + HDL-C + TG + Glucose + WC	−0.03 (0.50)	0.64	1.00	0.88	0.95	0.91	0.89	1									
8	MAP + HDL-C + TG + HOMA + WC + TC	−0.02 (0.47)	0.76	0.85	0.90	0.90	0.87	0.92	0.85	1								
9	MAP + HDL-C + TC:HDL-C + TG + Glucose + S4S	−0.01 (0.54)	0.74	0.93	0.91	0.89	0.86	0.87	0.93	0.86	1							
10	SBP + TC:HDL-C + Insulin	−0.01 (0.64)	0.66	0.69	0.80	0.79	0.68	0.83	0.66	0.86	0.71	1						
11	SBP + HDL-C + TG + Insulin + BMI	−0.05 (0.55)	0.53	0.83	0.94	0.90	0.86	0.97	0.81	0.89	0.81	0.88	1					
12	SBP + TC:HDL-C + TG + HOMA + WC	−0.05 (0.56)	0.65	0.84	0.91	0.91	0.85	0.93	0.83	0.97	0.83	0.91	0.94	1				
13	SBP + TC:HDL-C + TG + HOMA + S4S	−0.02 (0.59)	0.64	0.82	0.95	0.88	0.81	0.91	0.81	0.94	0.88	0.88	0.92	0.96	1			
14	SBP + TC:HDL-C + TG + HOMA + WC + CRF	−0.08 (0.54)	0.58	0.80	0.89	0.87	0.81	0.90	0.79	0.93	0.80	0.86	0.92	0.96	0.94	1		
15	SBP + TC:HDL-C + TG + HOMA + S4S + CRF	−0.05 (0.57)	0.57	0.78	0.92	0.84	0.77	0.88	0.76	0.89	0.84	0.83	0.89	0.92	0.96	0.97	1	
16	SBP + DBP + TG + HDL-C + Glucose + Insulin + BMI + WC + S4S	−0.01 (0.50)	0.57	0.91	0.93	0.93	0.86	0.91	0.91	0.86	0.86	0.74	0.87	0.87	0.89	0.87	0.87	1
Cluster scores	**Boys**																	
1	DBP + TC + HDL-C	0.03 (0.51)	1															
2	Average (SBP + DBP) + HDL-C + TG + Glucose + WC	−0.03 (0.50)	0.62	1														
3	Average (SBP + DBP) + HDL-C + TG + HOMA + S4S	−0.03 (0.55)	0.60	0.86	1													
4	Average (SBP + DBP) + HDL-C + TG + Glucose + Insulin + WC	−0.02 (0.50)	0.56	0.96	0.92	1												
5	MAP + HDL-C + TG + WC	−0.04 (0.53)	0.71	0.90	0.89	0.85	1											
6	MAP + HDL-C + TG + HOMA + BMI	−0.04 (0.52)	0.62	0.89	0.97	0.94	0.92	1										
7	MAP + HDL-C + TG + Glucose + WC	−0.02 (0.50)	0.64	1.00	0.86	0.95	0.90	0.88	1									
8	MAP + HDL-C + TG + HOMA + WC + TC	−0.04 (0.47)	0.75	0.84	0.91	0.90	0.87	0.93	0.84	1								
9	MAP + HDL-C + TC:HDL-C + TG + Glucose + S4S	−0.03 (0.53)	0.72	0.94	0.90	0.90	0.88	0.88	0.94	0.87	1							
10	SBP + TC:HDL-C + Insulin	−0.02 (0.64)	0.63	0.69	0.81	0.79	0.70	0.83	0.66	0.87	0.71	1						
11	SBP + HDL-C + TG + Insulin + BMI	−0.06 (0.56)	0.52	0.83	0.94	0.90	0.87	0.97	0.81	0.89	0.82	0.89	1					
12	SBP + TC:HDL-C + TG + HOMA + WC	−0.06 (0.56)	0.62	0.83	0.91	0.90	0.85	0.93	0.81	0.97	0.83	0.93	0.95	1				
13	SBP + TC:HDL-C + TG + HOMA + S4S	−0.06 (0.59)	0.61	0.80	0.95	0.87	0.82	0.91	0.79	0.94	0.86	0.90	0.93	0.97	1			
14	SBP + TC:HDL-C + TG + HOMA + WC + CRF	−0.10 (0.54)	0.56	0.78	0.89	0.86	0.81	0.90	0.76	0.92	0.80	0.87	0.92	0.96	0.95	1		
15	SBP + TC:HDL-C + TG + HOMA + S4S + CRF	−0.09 (0.57)	0.54	0.75	0.92	0.83	0.77	0.88	0.73	0.89	0.82	0.84	0.89	0.92	0.96	0.98	1	
16	SBP + DBP + TG + HDL-C + Glucose + Insulin + BMI + WC + S4S	−0.01 (0.50)	0.58	0.91	0.93	0.93	0.85	0.92	0.90	0.88	0.87	0.76	0.88	0.88	0.89	0.87	0.87	1

BMI: body mass index; CRF: cardiorespiratory fitness; HDL-C: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment; MAP: mean arterial pressure [DBP + (SBP-DBP/3)]; SD: standard deviation; S4S: sum of four skinfolds; TC: total cholesterol; TC:HDL-C: total cholesterol: high-density lipoprotein cholesterol ratio; TG: triglyceride; WC: waist circumference. *Log-transformed values:* WC, BMI, S4S, TG, TC:HDL-C, Insulin, and HOMA score.

[a] Clustered risk scores were constructed from examples of previously applied continuous cardiometabolic cluster scores in the pediatric population

[b] All correlations significant at the $p \leq 0.001$ level (2-tailed). In total, 6471 (girls n = 3230, boys n = 3241) had valid data in all risk variables.

Paper II

Cardiometabolic risk factor levels in Norwegian children compared to international reference values: The ASK study

Baseline characteristics of the ASK population are presented in **Table 4.** The population (N = 913) represent girls (n = 446) and boys (n = 467) having valid data in all cardiometabolic risk factors at baseline. There were no difference in mean age, height, BMI, WC, SBP, DBP or LDL-c between sexes. However, girls had significantly higher TC:HDL-c ratios, TG, insulin, and HOMA scores than boys, but lower CRF levels. Overall, girls had a less favorable risk score profile, represented by a higher clustered risk score than boys ($p < 0.001$).

Table 4. Baseline characteristics of the ASK study population by sex (paper II)

	Girls (*n* = 446)	**Boys** (*n* = 467)	
	Mean (±SD)/ median [Q1-Q3]/ n (%)	Mean (±SD)/ median [Q1-Q3]/n (%)	*p*-value
Age (yr)	10.2 (0.3)	10.2 (0.3)	0.885
Puberty (Tanner) n (%)			0.001
Stage 1	99 (22.2)	169 (36.2)	
Stage 2	292 (65.5)	248 (53.1)	
Stage 3-5	52 (11.7)	48 (10.3)	
Missing	3 (0.6)	2 (0.4)	
Parents` education level n (%)			0.888
≤ Upper secondary school	148 (33.2)	146 (31.3)	
<4 years of university	122 (27.4)	141 (30.2)	
≥4 years of university	159 (35.6)	159 (34.0)	
Missing	17 (3.8)	21 (4.5)	
Weight (kg)	37.1 (8.3)	37.0 (7.9)	0.941
Height (cm)	142.5 (6.8)	143.1 (6.7)	0.111
BMI (kg/m^2)	17.3 [15.9-19.6]	17.2 [15.8-19.4]	0.379
WC (cm)	59.6 [56.0-65.3]	60.8 [57.3-65.8]	0.061
SBP (mm Hg)	105.3 (8.5)	105.3 (8.2)	0.669
DBP (mm Hg)	58.1 (6.3)	57.4 (6.1)	0.095
LDL-c (mmol/L)	2.52 (0.62)	2.50 (0.67)	0.615
HDL-c (mmol/L)	1.55 (0.35)	1.63 (0.34)	0.001
TC:HDL-c ratio	2.82 [2.48-3.37]	2.72 [3.00-3.12]	0.001
Triglyceride (mmol/L)	0.73 [0.58-0.96]	0.65 [0.52-0.83]	<0.001
Glucose (mmol/L)	4.94 (0.33)	5.02 (0.32)	<0.001
Insulin (pmol/L)	52.8 [39.0-75.4]	45.4 [32.7-60.8]	<0.001
HOMA score	1.93 [1.37-2.83]	1.67 [1.19-2.29]	<0.001
Andersen test (m)	870.1 (84.7)	922.9 (111.7)	<0.001
Estimated VO_{2peak} (ml/kg/min)	50.5 (3.1)	57.1 (4.7)	<0.001
Clustered risk score	0.11 (0.68)	-0.10 (0.59)	<0.001

BMI: body mass index, CRF: cardiorespiratory fitness, DBP: diastolic blood pressure, HDL-c: high-density lipoprotein cholesterol, HOMA: homeostatic model assessment, LDL-c: low-density lipoprotein cholesterol, n: number, REF: (based on) reference values: SBP: systolic blood pressure, SD: standard deviation, TC: total cholesterol. A *p*-value ≤0.05 was considered statistically significant.

[a] The clustered risk score was calculated from the following risk variables SBP, ln (WC), ln (TC:HDL-c ratio), ln (TG), and ln (HOMA score) and CRF (inversed).

Figure 5 shows the standardized difference in single cardiometabolic risk factors and clustered risk scores between the Norwegian children and the international reference values. The Norwegian children had more favorable WC, DBP, glucose, HDL-C and CRF levels compared to the international reference population, but similar or less favorable levels of other cardiometabolic risk factors. Of all standardized variables, CRF differed the most from the international reference values, showing significantly more favorable levels in the Norwegian children (mean (95% CI) 1.20 (1.16 to 1.24) SD). The clustered risk score (excluding CRF) was higher in the Norwegian children (mean (95% CI) 0.15 (0.11 to 0.19) SD). On the contrary, when CRF was including, the clustered risk score decreased to below international levels (mean (95% CI) - 0.08 (- 0.12 to - 0.05) SD). Cardiorespiratory fitness had a significant inverse association with the clustered risk score (excluding CRF) (β - 0.37 SD, 95% CI - 0.43 to - 0.31). **Figure 6** illustrates the association between quartiles of CRF (quartile 1 represents the least fit children and quartile 4 the most fit children) and the reference-standardized clustered risk score by sex (*p* for trend < 0.001).

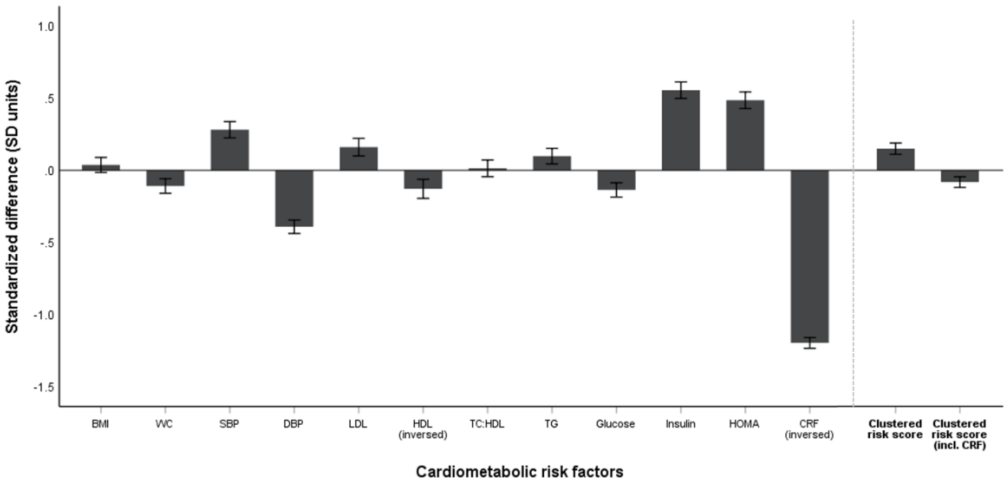

Figure 5. Reference-standardized cardiometabolic risk factors. Mean (95% CI) of the reference-standardized single risk factors and mean clustered risk scores excluding and including cardiorespiratory fitness (CRF) (inversed). Reference-standardized z-scores = x+x̄/sd (x̄), where age-predicted reference value was used as the mean (x̄) (199). The cardiometabolic clustered risk scores consisted of the following reference-standardized risk factors: SBP, ln (WC), ln (TG), ln (TC:HDL-c ratio), and ln (HOMA score), excluding and including CRF (inversed).

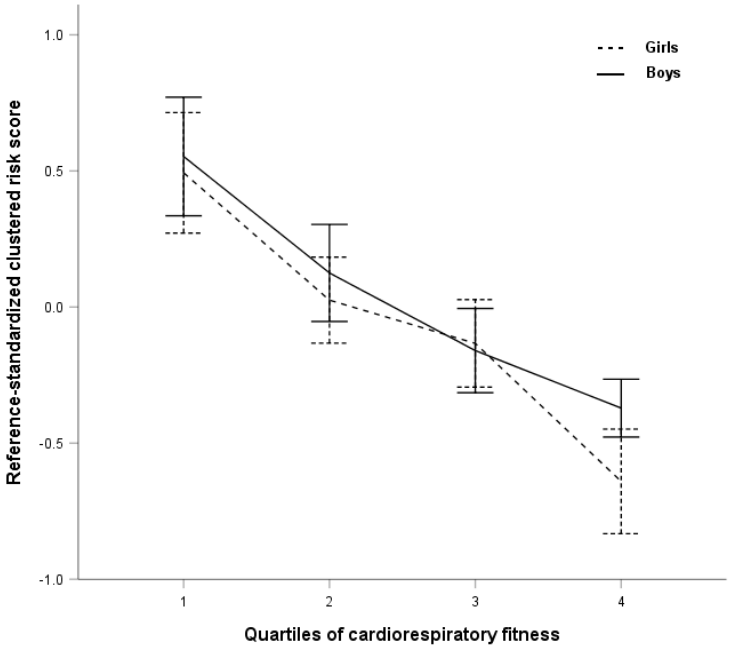

Figure 6. Association between quartiles of cardiorespiratory fitness and the reference-standardized clustered risk score by sex. Mean (95% CI) of the reference-standardized clustered cardiometabolic risk score (excluding CRF) across quartiles of CRF. A higher clustered risk score indicates a less favorable cardiometabolic profile. Children in quartile 1 of CRF are the least fit and children in quartile 4 the fittest. *P* for trend < 0.001.

Paper III

Effects of the Active Smarter Kids (ASK) physical activity intervention on cardiometabolic risk factors in children: a cluster-randomized controlled trial

Table 5. Baseline characteristics of intervention and control school children (paper III)

		Intervention		**Control**
	N	Mean (±SD)/ median [Q1-Q3]/ n (%)	N	Mean (±SD)/ median [Q1-Q3]/ n (%)
Age (yr)	596	10.2 (0.3)	533	10.2 (0.3)
Height (cm)	579	142.6 (6.8)	517	142.8 (6.8)
Weight (kg)	578	36.9 (8.0)	517	37.2 (8.1)
Puberty (tanner)	569		512	
Stage 1		170 (30)		139 (27)
Stage 2		330 (58)		318 (62)
Stage 3-5		69 (12)		55 (11)
Parents' education level	578		491	
≤ Upper secondary school		179 (31)		172 (35)
<4 years of university		179 (31)		142 (29)
≥4 years of university		220 (38)		177 (36)
Physical activity (school day)	538		538	
Total PA (cpm)		650 (186)		641 (192)
MVPA (min/day)		29 (11)		28 (10)
SED (min/day)		179 (20)		179 (21)
Physical activity (full day)	542		464	
Total PA (cpm)		745 (299)		723 (257)
MVPA (min/day)		77 (28)		74 (24)
SED (min/day)		468 (57)		468 (60)
% achieving PA guidelines		71		70
Cardiometabolic risk factors				
BMI (kg/m^2)	578	17.5 [15.9-19.5]	517	17.2 [16.0-19.6]
WC (cm)	577	60.3 [56.0-65.5]	517	60.3 [57.0-65.7]
SBP (mm Hg)	575	105.5 (8.3)	511	105.2 (8.7)
DBP (mm Hg)	575	58.2 (5.8)	511	57.5 (6.7)
LDL-c (mmol/L)	532	2.5 (0.7)	471	2.5 (0.6)
HDL-c (mmol/L)	533	1.6 (0.3)	471	1.6 (0.3)
Total cholesterol (mmol/L)	533	4.4 (0.7)	471	4.5 (0.7)
TC:HDL-c ratio	533	2.8 [2.4-3.3]	471	2.8 [2.4-3.2]
Triglyceride (mmol/L)	533	0.69 [0.55-0.89]	471	0.68 [0.55-0.89]
Glucose (mmol/L)	533	5.0 (0.3)	471	5.0 (0.3)
Insulin (pmol/L)	532	49.5 [35.3-68.2]	470	49.2 [35.1-66.8]
HOMA-score	532	1.8 [1.3-2.5]	470	1.8 [1.3-2.5]
Andersen (m)	550	893.6 (102.6)	495	891.9 (103.7)
Estimated VO_{2peak} (mL/kg/min)	549	54.2 (5.5)	495	54.0 (5.6)
Clustered risk score*	484	0.004 (0.66)	429	-0.005 (0.63)

BMI: body mass index: cpm: counts per min: CRF: cardiorespiratory fitness: DBP: diastolic blood pressure: HDL-c: high-density lipoprotein cholesterol: HOMA: homeostatic model assessment: LDL-c: low-density lipoprotein cholesterol: MVPA: moderate-to-vigorous physical activity: PA: physical activity: SBP: systolic blood pressure: SED: sedentary: TG: triglyceride. Risk variables in *italics* were skewed (nonnormal distribution). Values for PA were adjusted for valid wear time. PA guidelines represent children who achieved a mean minimum of 60 min/day of MVPA. *The clustered risk score was based on the following six cardiometabolic risk variables: WC, SBP, TC:HDL-c ratio, TG, HOMA-score, and CRF (inversed).

Baseline characteristic of intervention and control school children are presented in **Table 5**. There were no difference between intervention and control schools for any variable at baseline. On a group level, no intervention effects were found on single and clustered cardiometabolic risk factors (**Figure 7**). However, in children with the most unfavorable baseline values, effects were found in SBP (p < 0.1 for group*tertile interaction), TC:HDL-c ratio (p < 0.05 for group*tertile interaction) and the clustered cardiometabolic risk score (p < 0.05 for group*tertile interaction) (**Figure 8**). The effect of the intervention was also moderated by sex. Compared to boys, girls from intervention schools had more favorable effects on WC (p < 0.05 for group*sex interaction) and CRF (p < 0.001 for group*sex interaction) from baseline to follow-up (**Figure 9**). In stratified analysis, a significant intervention effect on CRF was found in girls (0.19 SD units, 95% CI 0.01-0.38).

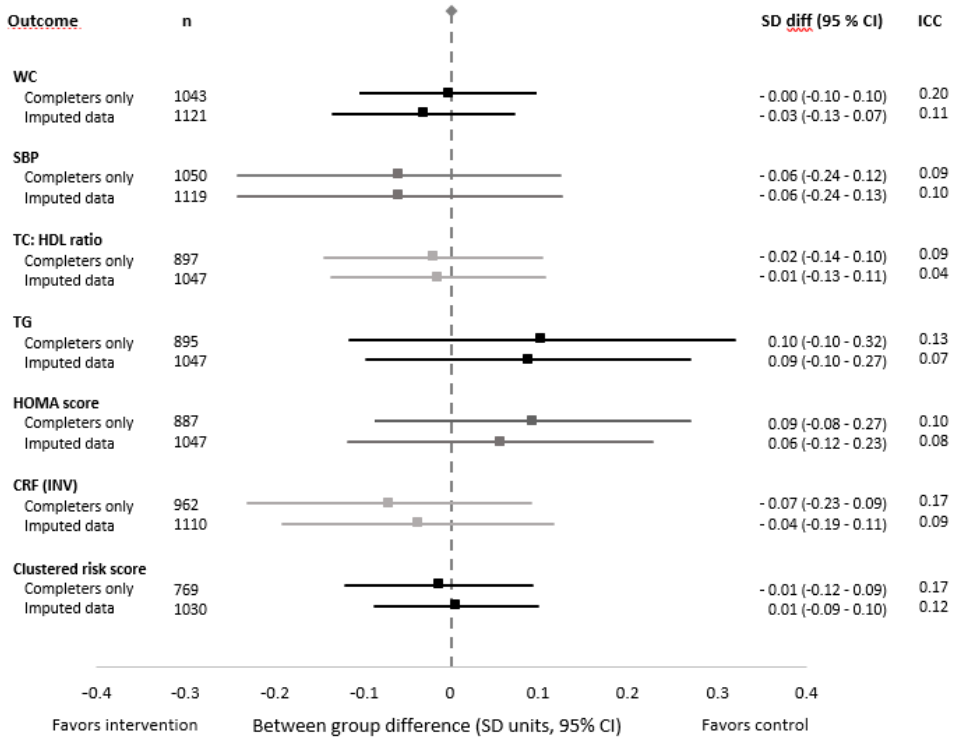

Figure 7. The intervention effect (intention-to-treat analyses) from completers only (n=769-1050) and from imputed data (N= 1030-1121) for those children having a valid measure at either baseline or follow-up. Effect sizes are presented in standardized units (SD) and 95% confidence interval. Cardiorespiratory fitness was inversed (CRF * -1).
95% CI; 95% confidence interval, HDL; high-density lipoprotein, HOMA; homeostatic model assessment, ICC; intraclass correlation coefficient (school), INV; inverse, SD; standardized difference, SBP; systolic blood pressure, TC; total cholesterol, TG; triglyceride, WC; waist circumference.

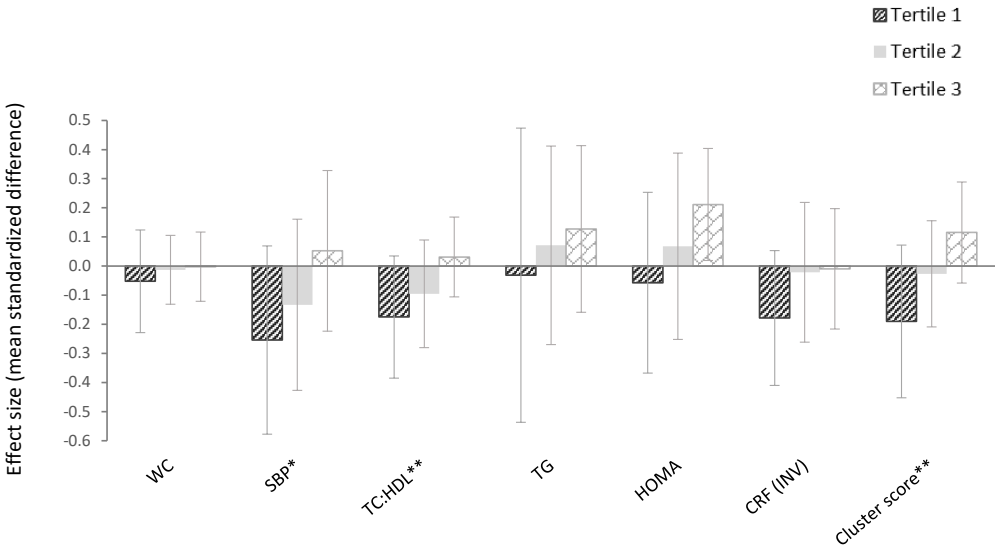

Figure 8. Subgroup differences between the intervention and control group by tertiles (tertile 1 is the least favorable group and tertile 3 is the most favorable group in the clustered risk score at baseline). Effect sizes are presented in standardized units (SD) ± 95% confidence intervals (CI). Subgroup consisted of n= 769 children having valid measures in all cardiometabolic risk variables. Cardiorespiratory fitness was inversed (CRF * -1). The clustered risk score consisted of the following variables: WC, SBP, TC:HDL-c ratio, TG, HOMA-score and CRF (inversed).
* Significant group x tertile interaction ($p < 0.1$). ** Significant group x tertile interaction ($p < 0.05$).

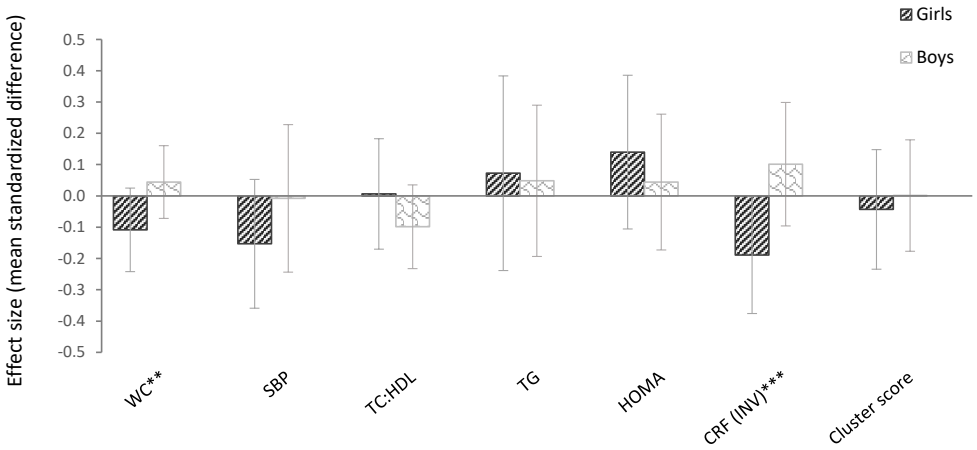

Figure 9. Subgroup differences between the intervention and control group by sex. Effect sizes are presented in standardized units (SD) ± 95% confidence intervals (CI). Subgroup consisted of n= 769 children having valid measures in all cardiometabolic risk variables. Cardiorespiratory fitness was inversed (CRF * -1). The clustered risk score was based on the following variables: WC, SBP, TC:HDL-c ratio, TG, HOMA-score and CRF (inversed).
* Significant group x sex interaction ($p < 0.1$). ** Significant group x sex interaction ($p < 0.05$). *** Significant group x sex interaction ($p < 0.001$).

Discussion

This thesis presents results from two study populations; 1) a pooled database from cohorts of children sampled from Northern, Southern, Mid and Eastern Europe and from the United States (**paper I**) and 2) Norwegian fifth-grade children participating in the ASK study **(paper II** and **paper III**).

Paper I presents the so far largest international reference values for cardiometabolic risk scores in children and adolescents aged 6 to 18 years, suggesting a common standard for defining cardiometabolic risk in children and adolescents. **Paper II** investigated the cardiometabolic risk levels in the group of Norwegian children participating in the ASK study by adapting the reference values from **paper I.** The study revealed that the Norwegian children had higher clustered risk scores than their international counterparts. However, the Norwegian children had significantly more favorable levels of CRF than the international children, and when CRF was including in the clustered risk score, risk score levels decreased to below international levels. Furthermore, cross-sectional analysis showed that CRF is associated with improved cardiometabolic health. **Paper III** investigated the effect of the ASK PA intervention on cardiometabolic risk factors and clustered cardiometabolic risk (including CRF). Overall, the PA intervention was not sufficient to significantly improve cardiometabolic risk factors in the total group of Norwegian children. Subgroup analysis, however, showed that girls had favorable effects on fitness and fatness, and the data suggests that children with the most unfavorable cardiometabolic risk profile at baseline can benefit from school-based PA interventions.

Reference values for cardiometabolic risk scores (paper I)

The present study provides reference values for cardiometabolic risk factors in a large sample of European and American 6- to 18-year-old children. Future studies investigating the prevalence of cardiometabolic risk in children or its associations with, for example lifestyle factors, may use the present data as a common reference when calculating and interpreting their results.

The reference values solve many of the identified obstacles when defining clustered cardiometabolic risk in children. For instance, no specific criteria, weighting of risk factors or thresholds are required when adapting the reference values to calculate clustered risk scores, hence, avoiding pitfalls such as selection bias and misclassification of individuals. Simultaneously, retaining variables continuous in clustered risk scores exploits all available information in the data. Additionally, the overall strong correlation found between different previously applied cardiometabolic risk scores in the pediatric population (**Table 3**), suggests that it is possible to compare cardiometabolic clustered risk scores

that include different ensembles of components. Thus, the reference values presented in **paper I** both offer an easy, transparent and flexible approach to standardize up to 14 different cardiometabolic risk variables, and also provide a meaningful fundament for comparison of clustered continuous risk scores in children. This unified approach thereby increases the prospects for estimating and comparing prevalence of and trends in cardiometabolic risk in children using continuous cardiometabolic risk scores, which can reduce confusion and allow for increased knowledge in this important field of research.

Three steps must be taken to calculate continuous reference-standardized clustered risk scores.

Step 1: Calculate individual age-and sex-predicted reference values for a given population by using the equation values (intercepts and beta-coefficients) provided in Table 2. The log-transformed values should be used for skewed variables.

Age-predicted reference values ($\bar{x}$) by sex:

$\bar{x}$ = γ-Intercept + βx *or*

$\bar{x}$ = γ-Intercept + ((βx) + (βx^2)) *or*

$\bar{x}$ = γ-Intercept + ((βx) + (βx^2) + (βx^3)),

where β = beta-coefficients (derived from Table 2) and x = age.

Example:

Reference values for systolic blood pressure for girls:

$\bar{x}$ = γ-Intercept + ((β-coefficient x) + (β-coefficient (x^2)) + (β-coefficient (x^3)))

$\bar{x}$ = 84.085419 + ((1.352664 age) + (0.100390 (age^2)) + (-0.005781 (age^3)))

Step 2: Calculate reference-standardized risk variables (z-scores) by using the age-predicted reference values (Step 1) and SD reference values derived from Table 3 via the following equation:

$$z\text{-score} = [(x - \bar{x})/SD(\bar{x})]$$

Example:

Standardized systolic blood pressure for girls:

$$z\text{-SBP} = (SBP - \bar{x})/8.6722095$$

Step 3: A mean clustered risk score can be derived using these z-scores by summing the variables of interest and dividing by the total number of variables included.

In the supplemental material for **paper I**, we provided a practical example of how to calculate reference-standardized clustered risk scores. In this example the following risk factors was used;

Mean clustered risk score = (z-SBP + z-(log)TC:HDL-c + z-(log)TG + z-(log)HOMA+ z-(log)WC)/5.

Paper II also serves as a practical example of how to use the reference values (via the three steps described above) and may provide inspiration for how to visualize and interpret the standardized single risk factors and clustered risk scores (results are discussed later).

Only a few studies offer the possibility to standardize pediatric cardiometabolic risk variables for use in epidemiological work and analysis (58, 205, 206). Reference values, but only for a single risk factors (SBP), were provided in the fourth report from the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents (205). Further, Gurka and colleagues (206) have suggested a standardized equation to calculate continuous risk scores. The equations are based on data from 4,174 American children aged 12–19 years participating in NHANES, comprising of five cardiometabolic risk variables: BMI, SBP, HDL-c, TG, and glucose. The approach provides gender- and racial/ethnic-specific risk scores, but does not consider age-related trends in cardiometabolic risk (207). Further, the equations are derived from confirmatory factor analyses. Factor analysis attempts to find latent variables by weighting single risk variables in a mathematical equation, but it depends on measurement error and short time fluctuations within each risk factor, rather than the contribution of risk itself. Finally, the ability of Gurka's clustered risk score to predict type 2 diabetes and carotid intima media thickness has been investigated, however, the score did not show a greater predictive utility compared to other continuous clustered risk scores (59). Andersen and colleagues have previously published reference values for single cardiometabolic risk variables, aiming to provide a basis for establishing the level of cardiometabolic risk in children (58). However, their study did not include log-transformed values for non-normally distributed variables, nor did it present common variability estimates presented, which are required for standardization of cardiometabolic risk factors and establishment of common reference values. The authors provide a software on the Internet to calculate risk scores useful in general practice. Although relevant for individuals the software does not offer a practical approach for larger population groups. Furthermore, the authors assumed linearity of all risk factors across the 12-year age span, thus ignoring possible non-linear trends caused by biological maturation (208, 209). The reference values presented in the present thesis, were based on the same data pooled by Andersen et al. (58). Yet, we extended the data set from 15,794 to 22,479 observations by including more recent survey releases from the NHANES. Thus, data are better balanced between European and US observations and cover the same time period during which the data from the European studies were obtained, while simultaneously taking the previous limitations of the study by Andersen et al. (58) into account.

The utility of cardiometabolic risk scores

The utility of the z-score approach to calculate clustered risk scores in pediatric epidemiological research increases substantially when sample-specific mean values are replaced with age-predicted reference values and SDs, as suggested in **paper I**. Using the reference material makes it possible to compare individual- and study-level data for single cardiometabolic risk variables and clustered risk scores to data from representative groups of European and American children and populations in studies adapting the same standardization strategy. The value of using the reference material will therefore increase with its usage in different studies and populations. The *true* utility of a cardiometabolic risk score lies, nonetheless, in its ability to estimate risk. In studies of pediatric cardiometabolic risk, continuous cluster risk scores have frequently been used (57, 59) and have been shown to be associated with levels of PA and CRF (112), and to predict adult type 2 diabetes and carotid artery intima media thickness (59). Comparison of the predictive ability of the continuous risk score approaches up against a dichotomized definition of the metabolic syndrome is less investigated. Studies do, nevertheless, show that the continuous risk score is either equal to (59) or superior to a dichotomized risk score for estimating children's future cardiovascular health and risk (210). More longitudinal studies that follow children over time are needed to illuminate this area of research, which could increase our knowledge of the best approach for predicting increased risk of cardiometabolic diseases from an early age.

Maturation and puberty

We investigated linear, quadratic, and cubic trends with age to fit the best equations for each of the 14 cardiometabolic risk variables. Consistent with previous studies, some risk factors did not change linearly over time and differed between sexes (208, 209, 211, 212), most likely because of biological changes related to age and maturation. These changes have been shown to influence the stability and accuracy of the metabolic syndrome diagnosis (207). For instance, puberty has a considerable impact on fat distribution, insulin sensitivity and secretion (211), and levels of TG have been shown to increase while levels of HDL-c decrease (208). Although we lack a complete pathophysiological understanding of all cardiometabolic risk factors, and the timing and influence of puberty and growth are unclear, we consider the use of non-linear trends in growing children an appropriate approach.

Race and ethnicity

Paper I did not provide race/ethnic-specific reference values. Among other reasons, presenting race/ethnic specific reference values would distract from the aim of **paper I:** to provide a *common* standard to define cardiometabolic risk. Making such subgroup-specific reference values would be

consistent with the current situation in the research field, in which continuous cardiometabolic risk scores are sample- (or subgroup-) specific. We do not consider sample diversity as a limitation for comparisons of standardized cardiometabolic risk scores. If risk factor levels differ among sub-groups, these groups will either have higher or lower standardized risk scores than other children, but still be comparable to the reference material and to other studies adapting the reference values for standardization on the same sub-group. Overall, about 60% of the sample population consisted of Caucasian children and the rest of other ethnic sub-groups: Hispanics, black-American, non-Hispanic blacks and "other race" (using the categories from the NHANES). However, some of these sub-groups are also broad and include several different ethnicities/races, for instance, the groups of non-Hispanic black and "other race". It is unlikely that isolated reference values for such subgroups would reveal more precise estimates of cardiometabolic risk. Thus, the reference values can arguably be perceived as representative for groups of children with diverse racial/ethnic origins. Although ethnic diversity might influence cardiometabolic risk factor levels (213), we argue that the proposed reference material in any case will facilitate comparison across studies and over time, and contribute to moving the research field of childhood cardiometabolic health forward.

Cardiometabolic risk levels in Norwegian children (paper II)

Adapting the reference values presented in **paper I** to standardize cardiometabolic risk factors in the ASK population showed that the Norwegian children had a higher clustered risk score of traditional risk factors than the international reference population, due to higher levels of SBP, HOMA score, and TG. However, CRF was considerably higher in the Norwegian children, and including CRF in the clustered risk score decreased the score to below international levels.

Systolic blood pressure

Systolic BP values in the children from the ASK study were significantly higher than the international reference values. Children from Nordic countries have earlier exhibited higher SBP values compared to children from other European countries (214, 215). Systolic BP was included in the clustered risk score, although DBP were lower in the Norwegian children compared to the international reference values. Systolic BP has been argued to be a recommended measure in children due to its greater accuracy and reproducibility than measures of DBP (216), and it is most frequently used as a single component to express BP levels in pediatric clustered risk scores (199). Dietary differences between countries could possibly impact on variations in SBP (217), but methodological differences could also be a plausible explanation for these findings. For instance, approximately 50% of the total population included in the reference material are drawn from the NHANES study, for which BP was measured by

the use of a mercury sphygmomanometer. The auscultation method using a mercury sphygmomanometer is the "gold standard" for BP measures (218). In the ASK study, an oscillometric BP device was used to measure BP. When validated against the mercury sphygmomanometer, oscillometric BP devices have been shown to overestimate SBP in children (2.53 mmHg; 95% CI [0.57-4.50]) (219). In comparison, the difference between SBP values in the Norwegian 10-year-olds compared to the international age-comparable children was 2.48 mmHg (95% CI [1.93-3.02]). On a positive note, although the Norwegian children exhibited higher SBP values than the international reference children, SBP levels seem to have decreased during the last decade among 9-10-year-old children from Sogn and Fjordane county (133).

Glucose, insulin levels and HOMA score

The ASK population had significantly higher HOMA scores than the international reference population. HOMA score has been shown to be a good surrogate measure for insulin resistance in youth, when validated against the euglycemic-hyperinsulinemic clamp method (220). Also, HOMA score has been shown to be a better predictor for clustered cardiometabolic risk factors in young adults than fasting glucose and insulin levels alone (221). During the last decades, prevalence rates of insulin resistance and type 2 diabetes in children and adolescents have been on the rise globally (49). Comparing HOMA levels in the ASK study to population-based samples of 9-10-year-old Norwegian children from 2005-2006 (177), shows that Norwegian children may be following international trends. Standardizing glucose and insulin separately according to the reference values showed that the ASK children had slightly lower glucose values (mean z = -0.13) but noticeably higher insulin values (mean z = 0.56) than the reference population. A potential methodological challenge when comparing insulin levels between populations is the use of different kits for analysis, since the binding characteristics of the plates may result in slightly different measurements. As an example of this, the EYHS study included in the reference material had their Danish blood samples analyzed shortly after collection in a WHO-certified laboratory. In contrast, the Portuguese blood samples were stored for several years before analysis. A subgroup of the Danish samples were reanalyzed to investigate the influence of the long storage time (almost 6 years), showing a strong correlation between insulin values between the first and second analysis (r = 0.97), but a substantial decrease (≈ 50%) in insulin levels. Thus, insulin levels from EYHS-Portugal were corrected for storage time to apply to all other obtained insulin values in the EYHS study. Nevertheless, it is plausible that storage time of blood samples in the different studies included in the reference material and in the ASK study contributes to the higher insulin values and HOMA score values observed.

Lipid and lipoproteins

Triglycerides and TC:HDL ratio were included in the clustered risk score in the present study to represent dyslipidemia, and both have been widely used in clustered risk scores in pediatric populations (199). Compared to the international reference population, TG levels were significantly higher in the Norwegian children. In stratified analysis, girls exhibited higher TG levels than the Norwegian boys. One explanation of this finding could be that TG concentrations are positively associated with sexual maturation (222), and girls have been shown to enter puberty at younger ages than observed previously (223). For instance, the Copenhagen Puberty Study found that thelarche among Danish girls in 2006 started nearly one year earlier than 15 years previously, independently of changes in BMI (224). The Norwegian children did not differ from the international reference population in TC:HDL-c ratio, despite higher HDL-c levels. High levels of HDL-c are positively correlated with CRF, which also was higher in the present study than the international reference values, and HDL-c has been shown to have an anti-atherosclerotic, and to some extent also cardioprotective, effect (225). Lipid and lipoproteins levels are, nonetheless, influenced by several environmental and genetic factors, such as diet (226) and apolipoprotein variants (227), which we were not able to control for.

Obesity

The Norwegian children had lower WC levels than the international reference values, despite that obesity levels have shown a significant linear increase in children and adolescents during recent decades in both Europe and the US (228, 229). In Europe, the prevalence of obesity has, nevertheless, not increased to the same extent as in the US. The WHO recently published a report (228), showing that the mean prevalence of obesity was 4% in 2014 among adolescents (11-, 13- and 15-year-olds) from more than 20 European countries. In comparison, the prevalence of obesity in 2014 was 20% among US children aged 12-15 years (229). However, the WHO reported that Norwegian adolescents had the lowest level of obesity in Europe in 2014, and Norway was one of the only European countries where an overall decrease in obesity was observed in the years between 2002 and 2014, although this decrease was significantly only in 13-year-old boys (228). In the ASK study, obese children accounted for < 4% using BMI cut-off points according to Cole et al. (230). The Norwegian children's BMI did not differ from the reference population, but WC was prioritized in the clustered risk score, since children's WC has been shown to be a stronger predictor of type 2 diabetes and more strongly associated with clustered cardiometabolic risk than is BMI (50, 231).

Cardiorespiratory fitness

Cardiorespiratory fitness was the variable that differed most from the international reference values. The ASK children had 1.20 and 1.23 higher SD values in girls and boys, respectively. When including CRF (inversed) in the clustered risk score, the risk score decreased considerably to below international standards. High levels of CRF in childhood have been associated with a healthier cardiometabolic profile later in life (139). In adults, CRF has been shown to be a stronger predictor of CVDs and all-cause mortality than other established risk factors, such as hypertension, type 2 diabetes and high cholesterol levels (25, 110-113). Furthermore, adding CRF to one or more of the traditional risk factors increases the predictive utility of adverse outcomes (113). Although different fitness tests were used to produce reference values for CRF, these fitness tests rely on solid validations to reflect absolute VO_{2peak} (199). In addition, VO_{2peak} levels in the ASK study were estimated from validated algorithms adapted from Aadland et al. (197). These algorithms were derived from age-comparable Norwegian children from Sogn and Fjordane, but could possibly have overestimated VO_{2peak} values. However, high CRF among Norwegian children is in line with findings from previous studies (133, 177). In addition, Norwegian children have been shown to be more physically active and active at higher intensities than children from most other countries in international comparisons (76).

Clustered cardiometabolic risk score

It is, nevertheless, rather contradictory that Norwegian children have higher SBP, TG and HOMA scores and consequently a higher clustered cardiometabolic risk score, despite lower WC and considerably higher CRF. One would expect lower (healthier) cardiometabolic risk factor levels in a fit population. However, similar findings were reported in the previous study by Resaland and colleagues among children from the Sogn and Fjordane county (133). The reason for the discrepancy in these risk factor profiles is difficult to identify, but it could be due to cultural or environmental factors or to any of the reasons discussed earlier, such as diet, genetics or methodological differences. The ASK population is both lean and fit, and despite having higher levels of some cardiometabolic risk factors compared to international values, these levels are still considered to be within a healthy range. Positively, general levels of the traditional risk factors as well as CRF seem to have improved in children from Western Norway during the last decade (133).

Association between cardiorespiratory fitness and clustered cardiometabolic risk

In accordance with earlier studies (93, 122, 133, 232, 233), **paper II** found an inverse association between CRF and clustered cardiometabolic risk (r = - 0.37). In comparison, Andersen et al. (232) found a stronger association between VO_{2peak} and clustered cardiometabolic risk in children (r = -

0.49) than was observed in the present study, while others have found a weaker association (*r* = - 0.31 to - 0.09) (122, 233). Aadland and colleagues (93) recently showed that CRF measured by the Andersen test is a more accurate marker of cardiometabolic health than is directly measured VO_{2peak} and time to exhaustion determined from a graded treadmill protocol in Norwegian 10-year-old children. The standardized regression coefficient between the Andersen test and clustered cardiometabolic risk in **paper II** was lower than that found by Aadland et al. (93); *r* = - 0.45. Still, the Andersen test in **paper II** performed slightly better than both VO_{2peak} and time to exhaustion presented by Aadland et al. (93) as a marker of cardiometabolic health.

As earlier mentioned, low CRF in adults has been shown to be one of the strongest determinants of overall mortality and risk of death caused by CVD, and thus poses a major challenge for public health (114, 234). In children, the evidence is less clear, but accumulating research shows an inverse relationship between levels of CRF and clustering of cardiometabolic risk factors (93, 122, 133, 232, 233). Cardiorespiratory fitness has traditionally not been included in the metabolic syndrome (or continuous clustered risks scores). Myers and colleagues have recently called CRF a generally overlooked and underutilized risk factor for cardiometabolic diseases, together with PA (112). The positive association of CRF with cardiometabolic health, however, strongly implicates its importance and argues for the inclusion of CRF in clustered cardiometabolic risk scores. Based on the same argument, CRF was included in the clustered risk score calculated in **paper III**.

Effects of the ASK intervention on cardiometabolic risk (paper III)

We found no significant intervention effect of the ASK school-based PA intervention on any of the cardiometabolic risk factors or the clustered risk score in either the intention-to-treat or the per-protocol analysis in the total population. We did, however, find significant moderation by baseline cardiometabolic risk on SBP, TC:HDL-c ratio and the clustered cardiometabolic risk score. Moreover, we found moderation by sex, showing that girls from the intervention schools had more favorable effects on WC and CRF compared to boys.

Effects of the intervention on the total population

There were no significant intervention effects on children's PA or sedentary behavior in the total sample from baseline to follow-up, measured objectively by accelerometers (230). High levels of PA in the control group might be an explanation for the lack of effect, which is not an unusual observation in PA intervention trials (235). Furthermore, the high baseline levels of PA in the ASK

study could indicate a potential ceiling effect in most children. ASK children from both the intervention schools and control schools were on average more physically active than population-based samples of 9-10-year-old Norwegian children (236), as well as broader samples of European and US children (76). Since the premise in the ASK study was that increased PA would cause a change in cardiometabolic risk factors (including CRF), this is likely to be the main reason why we were unable to detect measurable benefits on any of the cardiometabolic risk factors. Another reason could also be that the intervention potential, with respect to the cardiometabolic risk factors, was relatively low, as children generally have healthy cardiometabolic risk profiles (237). We also found no difference in cardiometabolic risk factor levels at follow-up between children from intervention and control schools in the per-protocol analysis (Appendix III, Table A.1). The per-protocol analysis excluded only one intervention school, while 13 control schools were excluded. It seems plausible that over-estimation of PA levels from intervention school teachers could have occurred, since no difference was found in accelerometer data (230).

PA interventions in school children have shown conflicting results with respect to effects on cardiometabolic risk factors, although with some indications of higher success in improving fitness levels (169, 170, 238, 239) rather than the traditional cardiometabolic risk factors of obesity, blood pressure, dyslipidemia, and insulin resistance (169, 238). Exploring intervention effects in generally healthy young children could, however, underestimate the true intervention effect. Some studies have found more beneficial effects among children with the most unfavorable baseline values in the clustered risk score and in CRF (201, 240). Resaland et al. (201) investigated the effect of a school-based PA intervention in 9-10-year-old Norwegian children, and found that children with the most unfavorable clustered risk profile at baseline benefitted the most. The same pattern was observed for the children with the lowest CRF at baseline (240).

Moderation by baseline values

Children in the ASK study with the most unfavorable baseline values in the clustered risk score (those in tertile 1) benefitted more from the PA intervention than children with more favorable values at baseline, supporting the findings from previous studies (201, 240). Although no stratified analyses of the intervention effect within the tertiles reached statistical significance, there was a consistent pattern of mean effect sizes in favor of the children in tertile 1. Post hoc analyses, investigating the moderating effect by tertile on accelerometer data measured mid-way in the ASK intervention period (Appendix III, Fig. A.1) showed a significant effect on all-day sedentary time in favor of tertile 1. Furthermore, in stratified analyses, children from intervention schools belonging to tertile 1 were significantly less sedentary during school hours and throughout the day than their peers from control

schools. Although no other analysis reached significance, the midway all-day PA levels (total PA, MVPA, and VPA) reflect the consistent pattern observed of changes in favor of the group of children having the least favorable cardiometabolic risk profile at baseline. In addition, comparing cardiometabolic risk factors levels with the international age- and sex-specific reference values from **paper I**, tertile 1 had a higher clustered risk score at baseline. The mean standardized difference in the clustered risk score (including CRF) was, nevertheless, only moderately higher (0.52 SD, 95% CI 0.47 to 0.58) than the international reference children, which could indicate that children, even with the most unfavorable risk profile, were within healthy cardiometabolic ranges and had a relatively low potential to change.

Moderation by sex

The subgroup analysis revealed a significant interaction effect by sex on WC and CRF, and girls from the intervention schools increased their CRF levels significantly more between baseline and follow-up than girls from control schools. Since girls' CRF levels have been shown to stagnate or even decrease after the age of nine (199), this is a positive outcome from the intervention. Sex-specific differences on outcomes in school-based PA interventions have been a controversial issue. However, a recent review of the effect of school-based RCT PA interventions on CRF showed a significant increase in CRF among girls, but not in boys (239). To better understand the different response in girls and boys observed in the present study, we performed post hoc analysis of the mid-way accelerometer data (Appendix III, Fig. A.2). The analysis showed significant interaction effects by sex on total PA levels, MVPA and VPA during school, and on all-day total PA levels and VPA levels in favor of the girls. Compared to girls from control schools, girls from intervention schools were significantly more physically active in total, did more MVPA and were less sedentary during school hours midway during the intervention. In accordance with studies showing that boys engage in more PA than girls (76), girls at baseline in the ASK study had significantly lower total PA levels and spent less time in MVPA and VPA both during school and throughout the day than the boys (Appendix III, Table A.2). In addition, girls had lower CRF levels and in general a less favorable risk profile than boys at baseline (**paper II**). Thus, girls had a larger potential for change as a response to the PA intervention than boys, which plausibly could explain the positive outcomes.

Methodological considerations

The application of different protocols and methodology when obtaining and handling data from cardiometabolic risk factors pose a challenge when comparing, synthesizing and pooling data from

different cohorts. In the following, some methodological considerations are made in regard to obtaining the cardiometabolic risk factors represented in this present thesis.

Blood pressure

The gold standard for indirect BP measurement has historically been the auscultation method using the mercury sphygmomanometer. However, in 2005 the WHO recommended a phase-out of mercury in the healthcare setting due to toxicity, leaving a lack of a common standard to measure BP in children (241). Mercury-free alternatives have been introduced, such as the android sphygmomanometer and automated oscillometric devices (242). Although the android sphygmomanometer has an aneroid pressure gauge instead of a mercury column, it still requires trained personal to measure BP by auscultation. Oscillometric devices have several practical features: first of all they are automated and do not require specialists, they have a good interrater reliability and have become the normally preferred BP monitor in both children and adults in research and clinical settings (242). To ensure that oscillimetric BP devices measure accurately in comparison to the mercury sphygmomanometer, validation protocols have been developed. The accuracy of oscillimetric BP devices are often validated against one or more of the following protocols: the British Hypertension Society protocol (BHS) (243), the International protocol (244) or Association for the Advancement of Medical Instrumentation (AAMI) protocol (245). However, only the International protocol for BP devices and the AAMI protocol have specific validation criteria for testing children.

Blood pressure measurements are relatively sensitive, and the accuracy can be influenced by several circumstances (246). These include environmental factors, such as the temperature of the room and possible disturbances, such as traffic noise or people entering the room (247). Blood pressure levels can also be affected by the behavior of the subject, such as PA or an uncomfortably full bladder, which are known to alter BP (248). The measurement protocol also has a significant impact on the accuracy of the BP measured, such as the detailed instructions of how the BP should be measured, the amount of time the subjects is to rest before the measurement, cuff size, posture of the subject (sitting or supine), crossed legs, support of the back and feet, which arm is used (left or right), placement of the cuff on the bare arm or over clothing, talking during the measurements, how many repeated measures will be taken, and how many and which measures will be used as the result (246). A high degree of standardization is possible to achieve for BP measurements across countries and time, but standardized measurements protocols are needed, combined with adequate training and monitoring during fieldwork and quality assessment (246).

In the reference material (**paper I**), the different studies included 5-15 minutes rest, and measures were taken in the left and/or right arm with 3-5 consecutive measurements at 1-2 minutes intervals.

In the analysis for **paper I**, three measures were summed and averaged. If >3 blood pressure measurements was taken, the last three measures were averaged and used. In the ASK study, the children rested for 10 minutes, four consecutive measurements were obtained in the right arm with 1-minute pauses in-between, and the mean of the last three measurements was used for analyses. If the difference between measurements was >5 mmHg, we obtained one extra measurement, in which case the mean of the last four measurements was calculated and used for analyses. All studies in the reference material (**paper I**) and the ASK study (**paper II** and **paper III**) used standard protocols, measures were taken in a sitting position, and appropriately sized cuffs were used for the individual child. All studies in the reference material used validated oscillometric BP devices. Further, the oscillometric BP device used in the ASK study (Omron HEM-907) has been validated against both the International protocol for BP devices and the AAMI protocol.

Waist circumference, body mass index and skinfold measures

The gold standard to obtain body obesity is currently the dual-energy X-ray absorptiometry (DXA) (249). Despite the high accuracy, DXA is not routinely used in clinical settings and epidemiology work since it is both costly and time consuming. Thus, several alternative methods have been suggested, such as measures of waist circumference, BMI and skinfold thickness, all of which have shown a good correlation to DXA fat mass in children (250).

Waist circumference is a simple and inexpensive clinical tool for measures of abdominal obesity, thus an excellent field test. The anatomical placement of the measuring tape, however, differs slightly between studies. A systematic review by Ross et al. (251) of 120 studies focusing on the anatomical site to obtain measures of WC showed that most studies measured WC at the midpoint between the lower rib margin and the iliac crest as suggested by WHO (252) (36%), the level of the umbilicus as suggested by the US National Institutes of Health (253) (28%), or the natural waist (25%) (251). The review observed similar patterns across sample size, sex, age, race and ethnicity in associations between all WC measurements and cardiometabolic risk, CVD and all-cause mortality (251). Further, the intra- and inter-tester reliability has been shown to be very high across these measurements (r = 0.97-0.99) (254). Importantly, the site does not appear to affect the relationship with visceral obesity (254). Reference values for WC should, however, optimally follow only one protocol. As recommended by the WHO, and the most used measure of WC in the literature, the midpoint was used to measure WC in CoSCIS (182), EYHS (178), and the NHANES (185) included in the reference material, thus representing > 80% of WC measurements in **paper I**. In addition, this was also the method used in the ASK study to assess WC (**paper II** and **paper III**). There are, however, also other methodological considerations when obtaining measures of WC - for instance, the type of tape used,

the tightness of the tape around the abdomen, the subject's posture, the phase of respiration, and abdominal tension (255).

Traditionally, BMI has been the preferred indicator of body composition and method to diagnose under- and overweight conditions (256), and like WC, it is a simple and inexpensive measure to obtain. Body mass index measures overall obesity calculated by a standard equation: body weight (kg) divided by height (m) squared. A few methodological considerations exist when obtaining measures to calculate BMI, such as the type of scale being used to obtain measures of body weight, since differences in accuracy have been observed between dial and digital scales showing that dial scales are less precise than digital scales (257), and considerations regarding clothing, such as whether the children are weighed dressed or undressed.

Skinfold thickness is a measure of the amount of subcutaneous fat, and the sum of skinfolds can be used to calculate body fat percentage with good precision (250). The inter-and intra-tester reliability have, nevertheless, been shown to be relatively low (< 0.85) in comparison to WC and BMI if not preceded by sufficient training (258, 259). The variation is often related to identification of the exact location for measurement, the way the skinfold is held, the way the calipers are placed on the fold, the compression of the fold by the calipers or the exact timing of the reading (260). Some of the studies included in the reference material (**paper I**), such as the EYHS study, investigated the inter- and intra-tester reliability for measures of skinfold thickness. EYHS found high inter-tester reliability (r = 0.84-0.99) and intra-tester reliability (r = 0.92-0.99) (178). Different sites have been recommended, but the four skinfolds taken from the biceps, triceps, subscapular, and suprailiac sites have been much used in epidemiological studies in children (59, 199) and are also the sites included in the reference material (**paper I**).

Lipids and lipoproteins

The standard lipid panel obtained in epidemiological studies and in clinical practice consists of measures of TG, TC, HDL-c and LDL-c. Some differences in protocols exist when obtaining and analyzing blood samples, for instance, in terms of requirements for fasting and whether venous or capillary blood is obtained.

In both epidemiological studies and clinical practice, fasting has traditionally been required before testing lipid levels, although we spent most of our time in non-fasting conditions. Studies have found that the risk associations of lipids with CVD are not weakened but may even be strengthened postprandial (261, 262), and the newest recommendations now allow for obtaining non-fasting lipid profiles for routine clinical practice (263, 264). This has practical implications for both patients with diabetes or other conditions that make it difficult to fast, and for children. In epidemiological

research, fasting values in children and young adults continue to be used, although some studies suggest that only small differences exist between fasting and non-fasting lipid panels in children, which may be clinically insignificant (186). However, for **paper I** we found it reasonable to correct non-fasting TC, HDL-c, and TG measures obtained for children aged six to 11 years in the NHANES for fasting time (hours) according to Steiner et al. (186), since the remaining studies included in the reference material had obtained only fasting blood samples. Steiner and colleagues (186) found that fasting time had a statistically significant negative effect on TG and a small but significant positive effect on TC and HDL-c. The study was based on children participating in the NHANES 1999–2008 surveys, corresponding to the surveys included in **paper I**.

It is well known that venous blood has different qualities than capillary blood - for instance, higher hemoglobin and hematocrit values (265). The reference values for lipids- and lipoprotein in **paper I,** as well as measures obtained in the ASK study **(paper II** and **paper III),** were all based on venous blood samples, except for the Norwegian part of the EYHS study, which used capillary blood samples to derive values of lipids and lipoproteins. Studies investigating differences in lipid and lipoprotein concentrations of capillary and venous blood samples (within-individual differences) are contradictory. Some studies find a statistical difference (266) while others do not (267, 268). No official recommendations are provided for the use of venous blood samples over capillary or against comparison (and pooling) of these, when measuring lipid and lipoprotein concentrations in children. Thus, we also included the measures of lipids and lipoproteins from the Norwegian part of the EYHS in **paper I**.

Insulin resistance

The euglycemic-hyperinsulinemic clamp technique is the gold standard for assessing insulin resistance in humans (269), but because it is time and financially consuming as well as laborious and more invasive than a single blood sample, the clamp technique is rarely used in clinical practice and epidemiological studies. Thus, fasting blood samples have been extensively used to measure insulin and glucose levels and calculate measures of insulin sensitivity, such as the HOMA score [insulin (pmol/L) * glucose (mmol/L)]/135 (188).

As discussed earlier, methodologically differences (such as the use of different kits for analysis) and storage time of blood samples can have a considerable impact on measured insulin levels. Also different methods for glucose determination can have an impact on obtained glucose levels, such as the use of different glucose-measuring platforms and systems for analysis. Furthermore, glucose levels vary depending on whether venous, capillary or arterial whole blood is obtained and used, and whether glucose concentrations are determined from plasma or serum samples (270). The

differences in plasma and serum glucose are not yet clearly understood, but some studies find higher levels of glucose in plasma than when analyzing serum (271). Further, postprandial capillary glucose levels can be up to 20% higher than in venous blood samples (272). All studies pooled for **paper I** and the ASK study (**paper II** and **paper III**) used venous serum samples. No data of glucose on insulin was included in **paper I** from the Norwegian part of the EYHS, which obtained capillary blood samples. Additionally, no data on glucose and insulin levels for American children between six and 11 years of age was included, since this age group had not been instructed to fast before blood sampling in NHANES.

Cardiorespiratory fitness

As earlier described, the gold standard for obtaining CRF is direct measures of VO_{2peak} from maximal exercise tests to exhaustion.

The reference values (**paper I**) for CRF included VO_{2peak} values from two directly measured VO_{2peak} tests: from a graded maximal treadmill running test and from a graded maximal bicycle ergometer. Five to 10% higher VO_{2peak} values are often expected in running performance tests compared to bicycle ergometer tests in children (86, 191, 273), since a larger muscle mass is utilized when running. The difference in VO_{2peak} must be taken into account when comparing studies using different test protocols or when pooling data. In **paper I**, results obtained from the cycle ergometer tests were corrected to comply with this issue. It is, nevertheless, possible that a correction of 5% did not adjust VO_{2peak} levels sufficiently.

Measures of direct VO_{2peak} require expensive equipment, highly trained personnel and time-consuming procedures, which can be less than feasible in large epidemiological studies, and many children experience discomfort from exhaustion tests. Thus, both field running tests and submaximal exercise test to predict Vo_{2peak} may be preferable in larger population studies and when testing children (94).

Probably the most used indirect field test to determine VO_{2peak} levels in children and adolescents worldwide is the 20 MSSRT (274, 275). The 20 MSSRT in children has demonstrated strong-to-very-strong test-retest reliability (r =0.89-0.93) (87, 89) and moderate-to-strong validity (r = 0.66-0.84) (87, 90). **Paper I** included VO_{2peak} obtained from the 20 MSSRT, but the data did, nevertheless, constitute only a minor part of the reference material (≈ 6%). The ASK study (**paper II** and **paper III**) used another field based running test, the Andersen test (88). The test-retest reliability has been shown to be strong-to-very strong (r = 0.84-0.92) (88, 91, 92) and it has strong validity (r = 0.72-0.84) (88, 91, 92) in children, adolescents and young adults. Common for both field-running tests is that the tests are generally accepted as providing reliable and valid data on whole-group level in children but have

a larger degree of individual variability for estimates of VO_{2peak} (91, 276). As an example of the measurement precision of field-based CRF running tests in children, the Andersen test has an ICC of 0.84 (91), while the ICC for objectively measured PA is approximately 0.50 (123, 124).

In comparison to a maximal CRF test, a submaximal exercise test is less accurate and has shown some reproducibility limitations in adolescent population groups (97). **Paper I** included VO_{2peak} values from the NHANES study for adolescents, which is one of the largest datasets of CRF collected among youth (277). The accuracy of the NHANES submaximal running test to predict VO_{2peak} among adolescents has been examined only in a single study by Finn et al. (278) in 288 individuals (11-18 years) in combination with VO_2 measured to maximal effort. In boys, the predicted mean of 49.8 (±9.4) mL/kg/min was not significantly different from the measured VO_{2peak} of 50.8 (±7.7) mL/kg/min (p = 0.153). The mean estimate for girls was, however, significantly higher at 43.0 (±8.6) mL/kg/min than the measured VO_{2peak} of 41.7(±8.0) mL/kg/min (p = 0.039). Although the girls had higher predicted mean values, a residual difference of less than 2 mL/kg/min is within daily variances (279).

Pooling of different measurement protocols when aiming at producing reference values is a general limitation in **paper I**. Thus, differences in cardiometabolic risk levels when comparing data to the international reference values could reflect these differences in methodology. In regard to CRF, the reference material represents mean VO_{2peak} values from widely used and validated tests in the pediatric population, which makes an argument for pooling data. However, methodological differences must still be taken into account when standardizing CRF according to the reference material. It is worth mentioning that variances in measured VO_{2peak} or aerobic performance can also be influenced by factors other than methodology, such as physiological factors: e.g., diurnal differences (279); running economy/mechanical efficiency (280, 281); VO_2 kinetics (282); physical/anthropometric factors such as body fat and height (283); and psychosocial factors such as effort, motivation, and self-efficacy (284).

Clustered cardiometabolic risk scores

One of the main strengths of using clustered cardiometabolic risk scores in children is that clustered risk scores provide a more precise indication of the child's risk profile than a single risk factor (37, 58). Single risk factors are often weakly associated with later cardiometabolic diseases, and many single risk factors pose low immediate risk for children, as noted earlier. Further, a clustered risk score is less sensitive to variations in the single risk factors, which could be caused by physiological day-to-day fluctuations (37) and to some degree also by methodologically issues.

Clustered risk scores based on dichotomized risk variables have several limitations, including different weighting of risk variables, arbitrarily chosen cut-points in children, and different included

risk variables, as mentioned earlier. These issues increase the risk of misclassification as well as limiting generalizability and hampering comparability (285). The traditional definitions of the metabolic syndrome classifies individuals at risk only when thresholds are exceeded in three risk variables. Dichotomous risk scores thus repudiates that cardiometabolic risk likely exists as a spectrum, an idea supported by studies showing an increased risk for individuals as the number of risk variables with abnormal values increases (286, 287). Thus, it is possible that individuals who exhibit levels just above thresholds for the risk variables defining the metabolic syndrome but who have normal or low levels on the other variables have lower cardiometabolic risk than an individual with values just below thresholds in all included risk variables in the diagnosis (38). In general, the prevalence of children having the metabolic syndrome is relatively low (<4%) (288), which might reflect some of the issues of adapting (or using) adult criteria to define cardiometabolic risk in children. In addition, low prevalence makes it difficult to show associations between variables in logistic regressions because of reduced power (57). Another concern is the poor stability of pediatric dichotomous risk score diagnoses, especially as children enter puberty, bringing into question the clinical utility of these risk scores in children. For instance, the study by Goodman et al. (207) showed that only about half of children with the metabolic syndrome still had it 3 years later in adolescence. Subsequent studies, both short-term studies (289) and long-term cohort studies following youth into adulthood (210), have supported these findings.

Using clustered risk scores based on continuous variables has several strengths. However, especially four limitations of continuous clustered risk scores have been highlighted in the literature (57). *First*, a major limitation of clustered continuous risk scores in previous studies has been the use of sample-specific mean and SD values to standardize single cardiometabolic risk variables, thus resulting in sample-specific clustered risk scores. Unless the demographic characteristics, distribution of data, and central tendency and variability are equal between two studies, these risk scores would not be comparable between the studies (57). However, reference values providing common means and SDs, such as are provided in **paper I**, can solve this limitation of sample-specific values. *Second*, another limitation in the literature is the use of different combinations of variables in the cardiometabolic risk scores, as also identified in dichotomous cluster scores. The reference values allow for the standardization of up to 14 single risk factors and thus provide a solid basis for comparison of clustered risk scores regardless of the ensemble of components included. The overall strong correlation between different clustered risks scores (Table 3) show that there is a high degree of comparability when standardizing risk variables according to the reference values. This has practical implications, since future studies have the flexibility to calculate a clustered mean risk score of their own choice or available components and still be able to compare their clustered risk scores to other

studies adapting the same standardization strategy. Nonetheless, methodological differences, race/ethnicity, environmental factors and other circumstances that could possibly affect levels of clustered risk, should still be addressed. *Third*, many earlier studies have not taken age- or maturity-related variation into consideration in their clustered risk scores. **Paper I** has provided age-specific reference values to overcome this limitation. Also, by allowing the variables to fluctuate with age, we acknowledge the well-known non-linear trends in single risk factors caused by biological maturation (208, 209, 211, 212). Common sex-specific SD values were, nevertheless, provided for each risk factor in **paper I**. While residuals for most variables did not vary with age, residuals increased with age for the skewed variables: WC, BMI, skinfold thickness, insulin, and HOMA score. After log transformation, the variation of residuals was considerably less. In **Paper I,** we compared two standardized clustered risk scores (consisting of SBP and the log-transformed variables TC:HDL-c ratio, TG, HOMA score, and WC) using either constant or age-specific SD values within the youngest and oldest age groups, which showed similar results (ICC ≥ 0.99). We therefore chose to use the same robust SDs for all age groups to allow for a simpler application of algorithms. *Fourth*, continuous clustered risk scores are based on the assumption that each component is weighted equally in predicting cardiometabolic risk. This means that single risk factors, that could be more unfavorable in an individual's risk profile, could be camouflaged in a clustered risk score. Statistical procedures such as factor analysis have been suggested to handle this issue (290), however, such statistical approaches often introduce other limitations, as discussed earlier.

The optimal ensemble of cardiometabolic risk factors in clustered risk scores?

The optimal ensemble of risk variables in clustered risk scores is not fully known and is still debated in the literature, although most researchers in the field agree that the physiological risk components to consider are hypertension, obesity, dyslipidemia, and insulin resistance. **Paper I** (the practical example), **paper II** and **paper III** make use of the same traditional risk variables in the clustered risk score: SBP, WC, TG, HDL-ratio, and HOMA score. Cardiorespiratory fitness was additionally included in the clustered risk scores in **paper II** and **paper III**, due to the strong evidence of increased health benefits with higher CRF (47, 48, 113, 139).

The present thesis included measures of SBP to express BP levels in the clustered risk scores. As earlier noted, SBP has been recommended as a measure in children due to its greater accuracy and reproducibility than DBP (216). Furthermore, SBP is used more frequently than DBP to express BP levels in pediatric clustered risk scores (59, 199). Elevated SBP levels have been associated with organ damage in children (291) and track moderately into adulthood, increasing the risk of subclinical atherosclerosis (9). In adults, SBP has been shown to be a better predictor of cardiometabolic risk

than DBP (292), and the treatment of isolated systolic hypertension has effectively reduced morbid cardiovascular events and lowered mortality (293).

Waist circumference was chosen over both BMI and skinfold thickness in the clustered risk scores as a measure of obesity in the present thesis for a number of reasons. Waist circumference in children and adolescents has been shown to be a strong predictor of clustering of cardiometabolic risk factors and type 2 diabetes, and these associations seem to be stronger for WC than for BMI (50, 231). In adults, WC has been shown to be a strong predictor of both type 2 diabetes and CVD mortality, independent of other traditional cardiometabolic risk components such as blood pressure, insulin resistance and lipid levels (294, 295). Abdominal obesity has also been shown to be a better predictor of cardiometabolic risk than overall obesity measured by BMI in adults (296). In addition, the use of BMI in the pediatric population, especially in growing children, can be challenging since increases in BMI often reflect increases in fat-free mass rather than body fat (297). BMI has also widely been criticized for not considering body fat distribution (297). Measurement of skinfold thickness is less used than WC and BMI in pediatric clustered risk score (59, 199), probably due to the complexity of obtaining these measures.

Triglycerides and TC:HDL ratio are measures to determine dyslipidemia and both have a central role in the development of atherosclerosis and major CVD events (75). The TC:HDL ratio and especially TG are much used as expressions of childhood dyslipidemia in continuous clustered risk scores (59, 199).

The HOMA score was used in the present thesis as a measure of insulin resistance. The HOMA score has been validated against the euglycemic-hyperinsulinemic clamp technique and has shown to be a good surrogate measure in youth (220), as discussed earlier. Fasting glucose is typically normal in children, and studies of glycemic control show that children manage to regulate their blood sugar well despite severe insulin resistance (298). Furthermore, a study by Andersen et al. (221) has shown that HOMA score is more strongly associated with clustered cardiometabolic risk factors than the 2-hour oral glucose tolerance test, as well as fasting glucose and insulin. The HOMA-score has also been shown to be the most informative cholesterol-related index (299).

Cardiorespiratory fitness is not included in the description of the traditional cardiometabolic risk factors, as mentioned earlier, but may be considered as a *sine qua non* variable for both cardiometabolic risk and health. Especially in adults, there is strong evidence for such an argument. Summing up evidence from the literature presented earlier in this thesis, then low CRF has not only been shown to be a stronger predictor of CVDs and all-cause mortality than other traditional risk factors (25, 110-113), but evidence also shows that CRF in addition to these risk factors enhances the precision in predicting CVD morbidity and mortality (113). The evidence is less clear in children, and low CRF has been found to be only moderately associated with increased risk of the metabolic syndrome and arterial stiffness in adulthood (139). However, stronger evidence is found associating

high levels of CRF in childhood and adolescence with a healthier cardiometabolic risk profile later in life (139). In addition, a consistent inverse association between CRF and clustered cardiometabolic risk is found among children (48, 93, 122, 133, 232, 233). Thus, there is nothing that contradicts that CRF should be considered as an additional variable in pediatric clustered risk scores.

Methodological considerations in the ASK study

The recruitment process in the ASK study was comprehensive and successfully enrolled all 60 invited schools that fulfilled the initial inclusion criteria (≥ 7 pupils enrolled). Based on conceptions for successful implementation (171) and sustainability (172) of school-based research studies, the ASK study carried out a multiple-stage plan to anchor the project within many stakeholders in the school system by approaching politicians, educational authorities, county and municipal leaders, school principals and teachers. Meetings for parents/guardians were also conducted in the individual schools, providing a thorough description of the study (both oral and written), the aims, measurement protocol and procedures, and any possible hazards, discomfort, or inconvenience as well as addressing any questions raised (176). The ASK study was positively received and accepted on all levels.

Following randomization, three schools did, nonetheless, withdraw from participating in the ASK study. Thus, a final count of 57 schools participated (response rate: 96% of invited children and 82% of the total 10-year-old population in Sogn and Fjordane). Valid data was obtained from 1,129 children, n = 596 children from intervention schools and n = 533 children from control schools. Thus, the required sample size (n= 468 children in each group) was met for detecting significant effects in both the main outcome variable, academic performance, as well as for secondary outcomes, such as cardiometabolic risk factors (176). However, the sample size varied for **paper III**. In the intention-to-treat analysis, n > 1,030 observations were included after imputation of data, but only n > 769 children had complete data on all cardiometabolic risk factors of interest. Although the number of observations, and thus power, was slightly reduced by including only completers in the analysis, the completers-only and intention-to-treat analyses produced similar results.

The ASK intervention was conducted in a real-world setting and consisted of a curriculum-prescribed PA intervention delivered by classroom teachers, thus increasing the external validity of outcomes and the generalizability of the ASK intervention model to other schools. The content of the intervention was developed with inspiration from earlier studies (180), as well as in cooperation with teachers at the intervention schools. Three large pre-intervention seminars were held for the intervention school teachers, as were as two regional refreshing sessions during the intervention period. During the entire period, support was given to the intervention school teachers via email and

telephone. Teachers were also provided with access to a password protected website (www.askbasen.no) with videos and content on approximately 100 different intervention lessons, which the teachers helped expand throughout the intervention period. Thus, the ASK study aimed to educate, qualify and empower the teachers to conduct the intervention as well as stimulate a sense of ownership of the intervention at those schools, by acknowledging that the school teachers were the main facilitators or agents of the ASK intervention (300). Stimulating a sense of ownership among the schoolteachers did, however, provide the ASK research group with less control over the intervention. Consequently, it may have made content more varied between schools, challenging the internal validity of the study. However, we considered the teachers personal knowledge of the students and the individual school environment as important factors for conducting the intervention. Teacher-related barriers to successful implementation of school-based interventions have earlier been reported to include low levels of confidence in conducting the physical activities, lack of knowledge, and level of expertise or qualifications. In a recent report from the ASK study by Lerum et al. (301), the overall conclusion from the intervention school teachers was that they felt well prepared, supported and qualified to conduct the intervention. Some teachers did, however, find the seminars too theoretically heavy and redundant, but many highlighted that the practical part of the seminars were quite useful (301).

In regard to sustainability of the ASK intervention, approximately 81% of the intervention schools have continued with parts of the ASK content. One out of three teachers have continued with the weekly dose of the physical active learning component prescribed in the ASK study (≥ 90 minutes), and one out of two have maintained the dose of PA breaks during academic lessons (≥ 25 minutes). Few schools have, nevertheless, continued with the PA homework (19%), which also raised some challenges during the intervention period, since neither the teachers nor researchers had full control of what was actually done after school. Teachers report that the physically active lessons in particular provide them with a good pedagogical tool, as well as adding variety into the academic curriculum. They experience that students with low academic achievement particularly benefit from these lessons and have a greater connection to their peers (301). Thus, the positive influence on social engagement among students in the ASK study adds to teachers' experience from previous school-based studies of beneficial effects on student enjoyment, active engagement and attitude toward others, attention and readiness to learn, and academic skills (302-305).

Although the ASK study had a successful implementation and anchoring process and seems to have been successful in empowering and supporting the teachers to feel confident providing the intervention, no effects were found from any of the cardiometabolic risk factors on a whole-population level. The study lasted for seven months, which might have been insufficient time to

invoke positive changes in the cardiometabolic risk factors. Some researchers have suggested that a minimum of one year is required before an intervention is settled within the school framework (306). However, previous school-based PA studies have found positive effects in CRF (170) and in several cardiometabolic risk factors (169) after only three months. The initial high levels of PA among the ASK intervention-school children could indicate a potential ceiling effect in most children, as earlier stated. The ASK study found no significant difference between intervention and control schools in students' objectively measured PA or sedentary behavior (230). The explanation for this seems to be high levels of PA in the control group. As our premise was that PA would cause a change in cardiometabolic risk, this is likely to be the main reason we were unable to detect measurable benefits in intervention-schools compared to control schools. Yet, teacher-reports of PA indicated high adherence to the intervention and a clear contrast between the groups. Only one intervention school was excluded in the per-protocol analysis for not performing ≥ 80% of the prescribed PA. Although the ASK intervention seemed feasible to the schools, more than 13 control schools exhibited 120% or more of the weekly mandatory prescribed PA/PE. These contrasting results between subjective and objective measures of PA might be partly expected, as over-reporting of PA is common by self-report measures. In addition, some of the activities performed by the intervention schools (e.g., activities focusing on motor skills such as throwing, catching, balance or muscular strength) may be underestimated by the objective measurements. Overall, the ASK study can be viewed as a study of the effect of PA without academic content vs. PA with academic content (230). The ASK intervention, which was designed to first and foremost effect academic performance, may have been insufficient with respect to the total daily dose of PA (frequency, intensity and duration) to positively effect cardiometabolic risk factors.

Due to the nature of the experiment, blinding of children and schools was not possible. Internally in the project, only the project management group had formal knowledge of group assignment. Both the data manager and statisticians were blinded to group allocation until analyses were conducted (176). In the present thesis, only one paper (**paper III**) investigated the effect of the ASK intervention, despite the strong experimental design. **Paper II** used baseline data to investigate cardiometabolic risk levels in Norwegian children, and also included a cross-sectional analysis of the relationship between CRF and clustered cardiometabolic risk. Cross-sectional studies have, nevertheless, the limitation of not investigating causality and are sensitive to confounding (see *Analysis*).

Analysis

All papers in the present thesis used linear mixed models, including a random intercept accounting for the cluster effect of cohort (**paper I**) or school **(paper II** and **paper III**). Linear mixed models are an

extension of simple linear regression that allow for both fixed and random effects, taking hierarchical structures into account. When accounting for clustering, different intercepts were estimated for each cluster included in the analysis. Adjusting for clustering is based on the assumption that the characteristics within clusters are more strongly correlated than between clusters. The ICC is an indication of the correlation of observations within the clusters and is calculated as the variance of intercepts divided by total error variance. A low ICC simply means clustering is low. In real-life cross sectional studies, the ICC are often expected to be ≤ 0.20 (204), which was observed in the present thesis. When randomizing clusters to either intervention or control groups, as done in the ASK study, clustering leads to loss of power and needs to be accounted for in the sample size calculation. In the ASK study, the design effect was estimated to be 4.54. Results on academic performance showed the assumed ICC (0.15) was correct (230). However, ICCs were generally lower for cardiometabolic risk factors, thus, these analyses (**paper III**) had high power.

The analyses of the effectiveness of the ASK intervention on cardiometabolic risk (**paper III**) were performed according to the statistical analysis plan (176). First, we performed intention-to-treat analysis. Missing data were imputed using the Markov Chain Monte Carlo procedure (with 20 iterations), since intention-to-treat analysis requires a complete dataset. The imputation methods are used to produce conservative estimates of intervention effects, although no imputation method can provide unbiased estimates. In accordance with the Consolidation Standard of Reporting Trials (CONSORT) guidelines for RCTs, flow charts are presented in **paper II** and **paper III** with information on missing data. Similar effects were found for the effect analysis in **paper III** of completers only (children having valid data in each cardiometabolic risk variable at both baseline and follow-up) and with imputed data. Second, we performed per-protocol analyses in **paper III**. In these analyses, 13 control schools were excluded, while only 1 intervention school did not follow intervention protocol. The large number of control schools excluded from the analysis demonstrates that it was not possible to restrict control schools to the mandatory weekly amount of PA and PE. Although this could be a reasonable explanation for the non-significant intervention effects found in the intention-to-treat analysis, the effect sizes were similar in the per-protocol analysis. Finally, we investigated the moderating effect of baseline cardiometabolic risk and sex. This was based on earlier studies finding moderation by baseline values of clustered risk (201). We hypothesized that intervention effects might be higher in girls, since girls typically have lower levels of MVPA (76) and CRF (203) than boys, which was also found in the ASK study (**paper II** and **paper III**). Girls may therefore have a higher intervention potential than boys. Effect modification was investigated by including interaction terms in the statistical model, by a multiplication between the main determinant and the potential effect modifier. A challenge of sub-group analysis is to produce significant results, since the sample size

within each subgroup, and thus statistical power, are reduced. Thus, a more liberal statistical significance level is often chosen when evaluating interaction terms, for example, by accepting *p*-values <0.1 (204) as we did in **paper III**.

In **paper II**, investigating the association between CRF and clustered cardiometabolic risk at baseline, we investigated confounding by age, sex, pubertal stage and socioeconomic status in the statistical model. Several studies have shown that these covariates are related to both CRF and clustered cardiometabolic risk: age (3, 33, 34, 76), sex (76, 209), pubertal stage (209, 307) and socioeconomic status (308-310). However, only sex and pubertal stage significantly changed the estimates and were therefore included in the model. Pubertal stage was self-reported by the children, and we acknowledge that relying on children's own perception of their maturity stage can introduce subjective errors. Another limitation of pubertal stage is that the Tanner stages measure only external signs of physical development. When comparing Tanner stages to physical examination, several previous studies have found a moderate (kappa (κ) ≈ 0.50) (311, 312) to excellent (κ ≥0.70) (313-315) agreement. The interaction effect of sex was also investigated, but in line with previous studies (122, 133), similar trends in the association between CRF and the clustered risk score were found in girls and boys, thus the results were reported for girls and boys jointly.

Strengths and limitations

Strengths

The main strengths in **paper I** are the large international population of children and the wide age-spectrum. The reference material and equations values provided in **paper I** extend previous work. The regression equations are provided for 14 of the most used single cardiometabolic risk factors in cardiometabolic risk scores and offer an easy, transparent and flexible approach to standardizing cardiometabolic risk variables for use in monitoring, profiling, and surveillance of CVD risk in children during growth and maturation. Tables of normative risk factor values by sex and age are additionally included in **paper I**, which also provide a fast means for evaluating levels of single risk factors, for instance, for clinicians.

The main strength of **paper II** is the use of the international reference values (**paper I**) to standardize cardiometabolic risk variables obtained in the ASK study. Another strength is that the study, in addition to the reference values, provides future studies with the possibility of comparing cardiometabolic risk levels to those found in the ASK study, if these studies adapt the same standardization strategy. Thus, the strength of the study can increase with the usage of the reference

values in different studies and populations. Furthermore, the study also makes it possible to look at secular trends in Norwegian children's clustered cardiometabolic risk in future studies.

A major strength in **paper III i**s the cluster RCT design of the ASK study. The ASK study was conducted in a real-world setting and consisted of a curriculum-prescribed PA intervention delivered by classroom teachers, which increases the external validity of the study. According to teacher reports, the implementation of the intervention seemed successful. Other strengths are the objectively measured PA, since accelerometers are considered optimal for quantifying the amount and intensity of PA, although some methodological issues exist, such as the use of different cut-points for intensity levels and the low sensitivity for obtaining measures from a range of activities.

Both **paper II** and **paper III** included a relatively large sample size and the ASK study had a low attrition rate, which decreases the risk of bias.

Limitations

In **paper I**, the data was restricted by the original study designs and study protocols. In general, methodologically differences pose a limitation when comparing, synthesizing and pooling data from different studies, as discussed earlier. Currently, there do not exist official standards for how to obtain and handle data for most cardiometabolic risk variables. The reference values represent mean values from some of the most used methods for obtaining cardiometabolic risk variables in the literature. The reference values do, nevertheless, not indicate desired levels of cardiometabolic risk but represent normative standards from representative groups of children. None of the *emerging* risk factors were included in the reference material, such as inflammatory markers, due to either missing data or few observations in the original studies. This is a limitation, since the inflammatory state is a pathophysiological mechanism directly related to the atherosclerotic process and its complications (316). However, Andersen et al. (58), including approximately 70% of the same data presented in **paper I**, analyzed a clustered risk score with and without CRP but found no difference in the association with other health markers, such as CRF.

Paper II was based on cross-sectional data from the ASK study. Exposure and outcomes were measured within the same time frame, which precludes concerns about temporality and indication of causality. The use of the Andersen aerobic fitness test to estimate VO_{2peak} could be a limitation of our findings, since the Andersen test is an indirect measure of CRF. However, the test has shown both validity and reliability in the target age-group (91, 197). The Andersen test also performs better as a marker of cardiometabolic health than both the direct VO_{2peak} test and the TTE (93). Furthermore, this test is more feasible in large population studies than direct measurement of VO_{2peak}. The

Andersen test is a suitable aerobic fitness test in children because the intermittent running reflects children's natural running pattern and because it does not stigmatize children with a low CRF level.

In **paper III**, generalization of results should be done with some caution, since the population represents only one county in Norway. The ASK intervention was designed to first and foremost affect the main outcome - academic performance. The daily dose (frequency, intensity and duration) of PA during a limited intervention period of 7 months, may have been insufficient to positively affect cardiometabolic risk factors. Thus, future school-based PA intervention studies may consider to introducing a third arm existing of PA without (or with less) academic content. Furthermore, the weekly PA reports were done by the school teachers, which might introduce subjective errors into the per-protocol analyses. One of the main limitations was the generally low intervention potential due to initially high PA levels with most children already meeting the guidelines of at least 60 minutes of MVPA daily. The ASK study did not, for instance, include a registration of food intake, but the randomized design limits the possibility of any confounding.

Conclusions

In **paper I**, the so far largest international reference material of cardiometabolic disease risk factors for the pediatric population was presented. This approach makes continuous single risks factors and clustered cardiometabolic disease risk scores comparable to the reference material itself and comparable to cardiometabolic disease risk values in future studies that uses the same standardization strategy, which arguably, is a significant step forward in the field of pediatric epidemiology.

In **paper II**, the international reference values (from **paper I**) was used to standardize risk factor values in Norwegian 10-year-old children from the ASK-study. Norwegian children have more favorable WC, DBP, glucose, HDL-c and CRF levels compared to international reference values, but similar or less favorable levels of other cardiometabolic risk factors. The reference-standardized clustered risk score (without CRF) shows that Norwegian children have a more unfavorable risk score profile of traditional risk factors than international children. However, Norwegian children have substantially higher CRF levels, and including CRF in clustered risk scores reduces overall cardiometabolic risk in Norwegian children below that of international levels. Additionally, CRF was associated with cardiometabolic risk, with low fit children scoring significantly higher on clustered cardiometabolic risk factors than children with higher CRF levels.

In **paper III**, the effect of the daily ASK school-based PA intervention among 10-year-old children was investigated. The majority of the children had high PA levels, thus limiting the potential for change, and no effects of the ASK school-based PA intervention was found on single or clustered cardiometabolic risk factors in the total sample. However, subgroup analysis showed that girls had a more favorable effect on fatness and fitness than boys, and the data suggest that subgroups of children with the most unfavorable cardiometabolic risk profile and thus most in need of change can benefit from school-based PA interventions.

Perspectives and implications

The international reference values presented in this thesis, suggest a common standard for defining and comparing continuous cardiometabolic clustered risk scores in the pediatric population. The reference values extend previous work with the a purpose of providing common standards for cardiometabolic risk factors, such as the extensively used standards for BMI by Cole et al. (317), to define and compare overweight and fatness levels. However, international consensus on standard methods to measure cardiometabolic risk factors are a future challenge in this area of research. Further, pediatric reference values for cardiometabolic risk factors based on longitudinal observational studies are needed to better reveal the association of cardiometabolic risk factors with age and maturation.

With an emphasis on the findings of the ASK study in the present thesis, future school-based intervention studies with the aim of significantly affecting cardiometabolic risk factors should put effort into 1) a higher weekly and daily dose of total PA, 2) a higher weekly and daily dose of MVPA, and 3) separate PA levels between the intervention and control groups. Further, longitudinal studies with more than two time points are warranted to observe the long-term effects of increased PA in school on cardiometabolic health.

Nevertheless, the ASK study has shown that PA integrated in academic lessons is fully possible and is feasible in the Norwegian school setting. More than 80% of the earlier ASK intervention schools have continued with parts of the content from the ASK intervention. Teachers report that the physically active lessons in particular provide them with a good pedagogical tool and add variety into the academic school day for them and the students. The ASK study has shown that low academic achievers (230, 301), as well as those children with the most unfavorable cardiometabolic risk profile (**paper III**), seem to benefit the most from such school-based strategies. From this perspective, curriculum-prescribed PA could be a contributing mean to decrease polarization and inequality between students in the schools, regardless of their socioeconomic background or general health behavior. I do believe that this, together with the knowledge database of the multiple health benefits of general PA, provides the Norwegian government with a mandate to go through with the already existing plans for integrating more PA in the schools.

Although the school alone cannot solve the general problem of inactivity, initiatives to increase PA during school time do have the potential to ensure that students meet guidelines for recommended daily PA. Increased levels in cardiometabolic risk factors may provide an early warning of later cardiometabolic risk. Children with low CRF and low PA levels could especially be at risk of

developing later lifestyle related diseases. Thus, childhood, as a time in life when lifestyle-related changes might be most efficient, may also be a beneficial time for preventative action.

References

1. WHO. Global Health Estimates 2016: Disease burden by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organization. 2018.
2. Collaborators GBDCoD. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159):1736-88.
3. McGill HC, Jr., Herderick EE, McMahan CA, Zieske AW, Malcolm GT, Tracy RE, et al. Atherosclerosis in youth. Minerva Pediatr. 2002;54(5):437-47.
4. McGill HC, Jr., McMahan CA, Tracy RE, Oalmann MC, Cornhill JF, Herderick EE, et al. Relation of a postmortem renal index of hypertension to atherosclerosis and coronary artery size in young men and women. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb Vasc Biol. 1998;18(7):1108-18.
5. McGill HC, Jr., McMahan CA, Zieske AW, Sloop GD, Walcott JV, Troxclair DA, et al. Associations of coronary heart disease risk factors with the intermediate lesion of atherosclerosis in youth. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb Vasc Biol. 2000;20(8):1998-2004.
6. McGill HC, Jr., McMahan CA, Zieske AW, Tracy RE, Malcom GT, Herderick EE, et al. Association of Coronary Heart Disease Risk Factors with microscopic qualities of coronary atherosclerosis in youth. Circulation. 2000;102(4):374-9.
7. McGill HC, Jr., McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. Circulation. 2008;117(9):1216-27.
8. Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. Obes Rev. 2008;9(5):474-88.
9. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation. 2008;117(25):3171-80.
10. Nicklas TA, von Duvillard SP, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: the Bogalusa Heart Study. Int J Sports Med. 2002;23 Suppl 1:S39-43.
11. Twisk JW, Kemper HC, van Mechelen W, Post GB. Tracking of risk factors for coronary heart disease over a 14-year period: a comparison between lifestyle and biologic risk factors with data from the Amsterdam Growth and Health Study. Am J Epidemiol. 1997;145(10):888-98.
12. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. JAMA. 2003;290(17):2277-83.
13. Eisenmann JC, Welk GJ, Wickel EE, Blair SN, Aerobics Center Longitudinal S. Stability of variables associated with the metabolic syndrome from adolescence to adulthood: the Aerobics Center Longitudinal Study. Am J Hum Biol. 2004;16(6):690-6.
14. Chen W, Srinivasan SR, Li SX, Xu JH, Berenson GS. Clustering of long-term trends in metabolic syndrome variables from childhood to adulthood in blacks and whites - The Bogalusa Heart Study. Am J Epidemiol. 2007;166(5):527-33.
15. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
16. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539-53.

17. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C, American Heart A, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2004;109(3):433-8.
18. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366(9491):1059-62.
19. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004;350(23):2362-74.
20. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med. 2003;157(8):821-7.
21. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059-61.
22. Cohen J. The Cost of Dichotomization. Applied Psychological Measurement. 1983;7(3):249-53.
23. MacCallum RC, Zhang S, Preacher KJ, Rucker DD. On the practice of dichotomization of quantitative variables. Psychol Methods. 2002;7(1):19-40.
24. Beckstead JW, Beckie TM. How much information can metabolic syndrome provide? An application of information theory. Med Decis Making. 2011;31(1):79-92.
25. Blair SN. Physical inactivity: the biggest public health problem of the 21st century. Br J Sports Med. 2009;43(1):1-2.
26. Lee CD, Blair SN, Jackson AS. Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. Am J Clin Nutr. 1999;69(3):373-80.
27. WHO. Global Strategy on Diet, Physical Activity and Health. Geneva, World Health Organization. 2004.
28. Naylor PJ, McKay HA. Prevention in the first place: schools a setting for action on physical inactivity. Br J Sports Med. 2009;43(1):10-3.
29. Ogurtsova K, Fernandes JDDR, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pr. 2017;128:40-50.
30. Assoc AD. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2010;33:S62-S9.
31. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of Diabetes and Diabetes-Related Complications. Physical Therapy. 2008;88(11):1254-64.
32. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart Disease and Stroke Statistics-2017 Update A Report From the American Heart Association. Circulation. 2017;135(10):E146-E603.
33. Berenson GS, Wattigney WA, Tracy RE, Newman WP, 3rd, Srinivasan SR, Webber LS, et al. Atherosclerosis of the aorta and coronary arteries and cardiovascular risk factors in persons aged 6 to 30 years and studied at necropsy (The Bogalusa Heart Study). Am J Cardiol. 1992;70(9):851-8.
34. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med. 1998;338(23):1650-6.
35. Insull W, Jr. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. Am J Med. 2009;122(1 Suppl):S3-S14.
36. Andersen LB, Harro M, Sardinha LB, Froberg K, Ekelund U, Brage S, et al. Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). Lancet. 2006;368(9532):299-304.
37. Andersen LB, Wedderkopp N, Hansen HS, Cooper AR, Froberg K. Biological cardiovascular risk factors cluster in Danish children and adolescents: the European Youth Heart Study. Prev Med. 2003;37(4):363-7.
38. DeBoer MD, Gurka MJ. Clinical utility of metabolic syndrome severity scores: considerations for practitioners. Diabet Metab Synd Ob. 2017;10:65-72.

39. de Ferranti S, Mozaffarian D. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. Clin Chem. 2008;54(6):945-55.
40. DeBoer MD. Obesity, systemic inflammation, and increased risk for cardiovascular disease and diabetes among adolescents: a need for screening tools to target interventions. Nutrition. 2013;29(2):379-86.
41. Ford ES, Ajani UA, Mokdad AH, National H, Nutrition E. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. Diabetes Care. 2005;28(4):878-81.
42. Gagliardi ACM, Miname MH, Santos RD. Uric acid: A marker of increased cardiovascular risk. Atherosclerosis. 2009;202(1):11-7.
43. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, et al. Lipoprotein management in patients with cardiometabolic risk - Consensus conference report from the American diabetes association and the American college of cardiology foundation. Journal of the American College of Cardiology. 2008;51(15):1512-24.
44. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatr Diabetes. 2007;8(5):299-306.
45. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595-607.
46. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, et al. Lipoprotein management in patients with cardiometabolic risk. Diabetes Care. 2008;31(4):811-22.
47. McMurray RG, Ondrak KS. Cardiometabolic Risk Factors in Children: The Importance of Physical Activity. American Journal of Lifestyle Medicine. 2013;7(5):292-303.
48. Janssen I, LeBlanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int J Behav Nutr Phy. 2010;7.
49. Farsani SF, van der Aa MP, van der Vorst MMJ, Knibbe CAJ, de Boer A. Global trends in the incidence and prevalence of type 2 diabetes in children and adolescents: a systematic review and evaluation of methodological approaches. Diabetologia. 2013;56(7):1471-88.
50. Franks PW, Hanson RL, Knowler WC, Moffett C, Enos G, Infante AM, et al. Childhood predictors of young-onset type 2 diabetes. Diabetes. 2007;56(12):2964-72.
51. Morrison JA, Friedman LA, Wang P, Glueck CJ. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. J Pediatr. 2008;152(2):201-6.
52. Kylin E. Studien über das Hypertonie-Hyperglykämie-Hyperurikämie Syndrom [German]. Zentralblatt für Innere Medizin. 1923(44):105 –12.
53. Kahn R, Buse J, Ferrannini E, Stern M, American Diabetes A, European Association for the Study of D. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2005;28(9):2289-304.
54. Reinehr T, de Sousa G, Toschke AM, Andler W. Comparison of metabolic syndrome prevalence using eight different definitions: a critical approach. Arch Dis Child. 2007;92(12):1067-72.
55. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007;49(4):403-14.
56. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: The Princeton Lipid Research Clinics follow-up study. Pediatrics. 2007;120(2):340-5.
57. Eisenmann JC. On the use of a continuous metabolic syndrome score in pediatric research. Cardiovasc Diabetol. 2008;7:17.
58. Andersen LB, Lauersen JB, Brond JC, Anderssen SA, Sardinha LB, Steene-Johannessen J, et al. A new approach to define and diagnose cardiometabolic disorder in children. J Diabetes Res. 2015;2015:539835.

59. Magnussen CG, Cheriyan S, Sabin MA, Juonala M, Koskinen J, Thomson R, et al. Continuous and Dichotomous Metabolic Syndrome Definitions in Youth Predict Adult Type 2 Diabetes and Carotid Artery Intima Media Thickness: The Cardiovascular Risk in Young Finns Study. J Pediatr. 2016;171:97-103 e3.
60. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100(2):126-31.
61. Corder K, Ekelund U, Steele RM, Wareham NJ, Brage S. Assessment of physical activity in youth. J Appl Physiol. 2008;105(3):977-87.
62. Dollman J, Okely AD, Hardy L, Timperio A, Salmon J, Hills AP. A hitchhiker's guide to assessing young people's physical activity: Deciding what method to use. Journal of Science and Medicine in Sport. 2009;12(5):518-25.
63. Cain KL, Sallis JF, Conway TL, Van Dyck D, Calhoon L. Using accelerometers in youth physical activity studies: a review of methods. J Phys Act Health. 2013;10(3):437-50.
64. Welk GJ, Corbin CB, Dale D. Measurement issues in the assessment of physical activity in children. Res Q Exerc Sport. 2000;71(2 Suppl):S59-73.
65. Bailey RC, Olson J, Pepper SL, Porszasz J, Barstow TJ, Cooper DM. The Level and Tempo of Childrens Physical Activities - an Observational Study. Med Sci Sport Exer. 1995;27(7):1033-41.
66. Freedson P, Pober D, Janz KF. Calibration of accelerometer output for children. Med Sci Sport Exer. 2005;37(11):S523-S30.
67. Sallis JF, Saelens BE. Assessment of physical activity by self-report: status, limitations, and future directions. Res Q Exerc Sport. 2000;71 Suppl 2:1-14.
68. Sirard JR, Pate RR. Physical activity assessment in children and adolescents. Sports Med. 2001;31(6):439-54.
69. Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, et al. Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. Int J Behav Nutr Phys Act. 2017;14(1):75.
70. Troiano RP, McClain JJ, Brychta RJ, Chen KY. Evolution of accelerometer methods for physical activity research. Brit J Sport Med. 2014;48(13).
71. Corder K, Brage S, Wareham NJ, Ekelund U. Comparison of PAEE from combined and separate heart rate and movement models in children. Med Sci Sports Exerc. 2005;37(10):1761-7.
72. Ekelund U, Sjostrom M, Yngve A, Poortvliet E, Nilsson A, Froberg K, et al. Physical activity assessed by activity monitor and doubly labeled water in children. Med Sci Sports Exerc. 2001;33(2):275-81.
73. Trost SG, Ward DS, Moorehead SM, Watson PD, Riner W, Burke JR. Validity of the computer science and applications (CSA) activity monitor in children. Med Sci Sports Exerc. 1998;30(4):629-33.
74. Pate RR, Pfeiffer KA, Trost SG, Ziegler P, Dowda M. Physical activity among children attending preschools. Pediatrics. 2004;114(5):1258-63.
75. Brage S, Wedderkopp N, Franks PW, Andersen LB, Froberg K. Reexamination of validity and reliability of the CSA monitor in walking and running. Med Sci Sports Exerc. 2003;35(8):1447-54.
76. Cooper AR, Goodman A, Page AS, Sherar LB, Esliger DW, van Sluijs EMF, et al. Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). Int J Behav Nutr Phy. 2015;12.
77. Riddoch CJ, Bo Andersen L, Wedderkopp N, Harro M, Klasson-Heggebo L, Sardinha LB, et al. Physical activity levels and patterns of 9- and 15-yr-old European children. Med Sci Sports Exerc. 2004;36(1):86-92.
78. Malina RM. Physical activity and fitness: pathways from childhood to adulthood. Am J Hum Biol. 2001;13(2):162-72.
79. Raitakari OT, Porkka KV, Taimela S, Telama R, Rasanen L, Viikari JS. Effects of persistent physical activity and inactivity on coronary risk factors in children and young adults. The Cardiovascular Risk in Young Finns Study. Am J Epidemiol. 1994;140(3):195-205.
80. WHO. Global Recommendations on Physical Activity for Health. Geneva; World Health Organization. 2010.

81. Helsedirektoratet. Anbefalinger om kosthold, ernæring og fysisk aktivitet. 2014.
82. Shephard RJ, Allen C, Benade AJ, Davies CT, Di Prampero PE, Hedman R, et al. The maximum oxygen intake. An international reference standard of cardiorespiratory fitness. Bull World Health Organ. 1968;38(5):757-64.
83. Welsman J, Bywater K, Farr C, Welford D, Armstrong N. Reliability of peak VO2 and maximal cardiac output assessed using thoracic bioimpedance in children. Eur J Appl Physiol. 2005;94(3):228-34.
84. Rowland T. Children`s Exercise Physiology, 2nd ed. Champaign, IL: Human Kinetics. 2005.
85. Armstrong N, Kirby BJ, Mcmanus AM, Welsman JR. Aerobic Fitness of Prepubescent Children. Ann Hum Biol. 1995;22(5):427-41.
86. Armstrong N, Williams J, Balding J, Gentle P, Kirby B. The Peak Oxygen-Uptake of British Children with Reference to Age, Sex and Sexual Maturity. Eur J Appl Physiol. 1991;62(5):369-75.
87. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. J Sport Sci. 1988;6(2):93-101.
88. Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. J Sports Med Phys Fitness. 2008;48(4):434-7.
89. Liu NYS, Plowman SA, Looney MA. The Reliability and Validity of the 20-Meter Shuttle Test in American Students 12 to 15 Years Old. Res Q Exercise Sport. 1992;63(4):360-5.
90. Mayorga-Vega D, Aguilar-Soto P, Viciana J. Criterion-Related Validity of the 20-M Shuttle Run Test for Estimating Cardiorespiratory Fitness: A Meta-Analysis. J Sport Sci Med. 2015;14(3):536-47.
91. Aadland E, Terum T, Mamen A, Andersen LB, Resaland GK. The Andersen aerobic fitness test: reliability and validity in 10-year-old children. Plos One. 2014;9(10):e110492.
92. Ahler T, Bendiksen M, Krustrup P, Wedderkopp N. Aerobic fitness testing in 6- to 9-year-old children: reliability and validity of a modified Yo-Yo IR1 test and the Andersen test. Eur J Appl Physiol. 2012;112(3):871-6.
93. Aadland E, Kvalheim OM, Rajalahti T, Skrede T, Resaland GK. Aerobic fitness and metabolic health in children: A clinical validation of directly measured maximal oxygen consumption versus performance measures as markers of health. Prev Med Rep. 2017;7:74-6.
94. Noonan V, Dean E. Submaximal exercise testing: Clinical application and interpretation. Physical Therapy. 2000;80(8):782-807.
95. Montoye HJ, Ayen T, Washburn RA. The Estimation of Vo2max from Maximal and Sub-Maximal Measurements in Males, Age 10-39. Res Q Exercise Sport. 1986;57(3):250-3.
96. Åstrand PO. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. J Appl Physiol. 1954;7:218-21.
97. Andersen LB, Henckel P, Saltin B. Maximal oxygen uptake in Danish adolescents 16-19 years of age. Eur J Appl Physiol Occup Physiol. 1987;56(1):74-82.
98. Kasch FW. The Validity of the Astrand and Sjostrand Submaximal Tests. The Physician and Sportsmedicine. 1984;12(8):47-54.
99. Hartung GH, Blancq RJ, Lally DA, Krock LP. Estimation of Aerobic Capacity from Submaximal Ergometry in Women. Med Sci Sport Exer. 1995;27(3):452-7.
100. Kokkinos P, Myers J. Exercise and Physical Activity Clinical Outcomes and Applications. Circulation. 2010;122(16):1637-48.
101. Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW. Coronary heart-disease and physical activity of work. Lancet. 1953;265(6796):1111-20; concl.
102. Paffenbarger RS, Jr., Hyde RT, Wing AL, Lee IM, Jung DL, Kampert JB. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. N Engl J Med. 1993;328(8):538-45.
103. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events - Potential mediating mechanisms. Circulation. 2007;116(19):2110-8.
104. Kushi LH, Fee RM, Folsom AR, Mink PJ, Anderson KE, Sellers TA. Physical activity and mortality in postmenopausal women. Jama-J Am Med Assoc. 1997;277(16):1287-92.

105. Manson JE, Greenland P, LaCroix AZ, Stefanick ML, Mouton CP, Oberman A, et al. Walking compared with vigorous exercise for the prevention of cardiovascular events in women. New Engl J Med. 2002;347(10):716-25.
106. Gregg EW, Cauley JA, Stone K, Thompson TJ, Bauer DC, Cummings SR, et al. Relationship of changes in physical activity and mortality among older women. Jama-J Am Med Assoc. 2003;289(18):2379-86.
107. Paffenbarger RS, Hyde RT, Wing AL, Hsieh CC. Physical-Activity, All-Cause Mortality, and Longevity of College Alumni. New Engl J Med. 1986;314(10):605-13.
108. Kokkinos P, Myers J, Kokkinos JP, Pittaras A, Narayan P, Manolis A, et al. Exercise capacity and mortality in black and white men. Circulation. 2008;117(5):614-22.
109. Kujala UM, Kaprio J, Sarna S, Koskenvuo M. Relationship of leisure-time physical activity and mortality - The Finnish Twin Cohort. Jama-J Am Med Assoc. 1998;279(6):440-4.
110. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med. 2002;346(11):793-801.
111. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory Fitness as a Quantitative Predictor of All-Cause Mortality and Cardiovascular Events in Healthy Men and Women A Meta-analysis. Jama-J Am Med Assoc. 2009;301(19):2024-35.
112. Myers J, McAuley P, Lavie CJ, Despres JP, Arena R, Kokkinos P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status. Prog Cardiovasc Dis. 2015;57(4):306-14.
113. Ross R, Blair SN, Arena R, Church TS, Despres JP, Franklin BA, et al. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. Circulation. 2016;134(24):e653-e99.
114. Blair SN, Kampert JB, Kohl HW, 3rd, Barlow CE, Macera CA, Paffenbarger RS, Jr., et al. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. JAMA. 1996;276(3):205-10.
115. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH, Gibbons LW. Physical-Fitness and All-Cause Mortality - a Prospective-Study of Healthy-Men and Women. Jama-J Am Med Assoc. 1989;262(17):2395-401.
116. Lee DC, Artero EG, Sui XM, Blair SN. Mortality trends in the general population: the importance of cardiorespiratory fitness. J Psychopharmacol. 2010;24(11):27-35.
117. Blair SN, Kohl HW, 3rd, Barlow CE, Paffenbarger RS, Jr., Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. JAMA. 1995;273(14):1093-8.
118. Strong WB, Malina RM, Blimkie CJ, Daniels SR, Dishman RK, Gutin B, et al. Evidence based physical activity for school-age youth. J Pediatr. 2005;146(6):732-7.
119. Singh AS, Saliasi E, van den Berg V, Uijtdewilligen L, de Groot RHM, Jolles J, et al. Effects of physical activity interventions on cognitive and academic performance in children and adolescents: a novel combination of a systematic review and recommendations from an expert panel. Br J Sports Med. 2018.
120. Caspersen CJ, Nixon PA, DuRant RH. Physical activity epidemiology applied to children and adolescents. Exercise Sport Sci R. 1998;26:341-403.
121. Brage S, Wedderkopp N, Ekelund U, Franks PW, Wareham NJ, Andersen LB, et al. Objectively measured physical activity correlates with indices of insulin resistance in Danish children. The European Youth Heart Study (EYHS). Int J Obesity. 2004;28(11):1503-8.
122. Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S, et al. Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. Diabetologia. 2007;50(9):1832-40.
123. Mattocks C, Leary S, Ness A, Deere K, Saunders J, Kirkby J, et al. Intraindividual variation of objectively measured physical activity in children. Med Sci Sport Exer. 2007;39(4):622-9.

124. Aadland E, Andersen LB, Skrede T, Ekelund U, Anderssen SA, Resaland GK. Reproducibility of objectively measured physical activity and sedentary time over two seasons in children; Comparing a day-by-day and a week-by-week approach. Plos One. 2017;12(12).
125. Ekelund U. Cardiorespiratory fitness, exercise capacity and physical activity in children: are we measuring the right thing? Arch Dis Child. 2008;93(6):455-6.
126. Tell GS, Vellar OD. Physical fitness, physical activity, and cardiovascular disease risk factors in adolescents: the Oslo Youth Study. Prev Med. 1988;17(1):12-24.
127. Klasson-Heggebo L, Andersen LB, Wennlof AH, Sardinha LB, Harro M, Froberg K, et al. Graded associations between cardiorespiratory fitness, fatness, and blood pressure in children and adolescents. Br J Sports Med. 2006;40(1):25-9; discussion -9.
128. Brage S, Wedderkopp N, Ekelund U, Franks PW, Wareham NJ, Andersen LB, et al. Features of the metabolic syndrome are associated with objectively measured physical activity and fitness in Danish children: the European Youth Heart Study (EYHS). Diabetes Care. 2004;27(9):2141-8.
129. Rizzo NS, Ruiz JR, Hurtig-Wennlof A, Ortega FB, Sjostrom M. Relationship of physical activity, fitness, and fatness with clustered metabolic risk in children and adolescents: The European Youth Heart Study. J Pediatr-Us. 2007;150(4):388-94.
130. Kriemler S, Manser-Wenger S, Zahner L, Braun-Fahrlander C, Schindler C, Puder JJ. Reduced cardiorespiratory fitness, low physical activity and an urban environment are independently associated with increased cardiovascular risk in children. Diabetologia. 2008;51(8):1408-15.
131. Anderssen SA, Cooper AR, Riddoch C, Sardinha LB, Harro M, Brage S, et al. Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. European Journal of Cardiovascular Prevention & Rehabilitation. 2007;14(4):526-31.
132. Ruiz JR, Ortega FB, Rizzo NS, Villa I, Hurtig-Wennlof A, Oja L, et al. High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. Pediatr Res. 2007;61(3):350-5.
133. Resaland GK, Mamen A, Boreham C, Anderssen SA, Andersen LB. Cardiovascular risk factor clustering and its association with fitness in nine-year-old rural Norwegian children. Scand J Med Sci Sports. 2010;20(1):e112-20.
134. Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput JP, Janssen I, et al. Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. Appl Physiol Nutr Me. 2016;41(6):S197-S239.
135. Tarp J, Child A, White T, Westgate K, Bugge A, Grontved A, et al. Physical activity intensity, bout-duration, and cardiometabolic risk markers in children and adolescents. Int J Obesity. 2018;42(9):1639-50.
136. Telama R. Tracking of Physical Activity from Childhood to Adulthood: A Review. Obesity Facts. 2009;2(3):187-95.
137. Telama R, Yang XL, Leskinen E, Kankaanpaa A, Hirvensalo M, Tammelin T, et al. Tracking of Physical Activity from Early Childhood through Youth into Adulthood. Med Sci Sport Exer. 2014;46(5):955-62.
138. Palve KS, Pahkala K, Magnussen CG, Koivistoinen T, Juonala M, Kahonen M, et al. Association of Physical Activity in Childhood and Early Adulthood With Carotid Artery Elasticity 21 Years Later: The Cardiovascular Risk in Young Finns Study. J Am Heart Assoc. 2014;3(2).
139. Ruiz JR, Castro-Pinero J, Artero EG, Ortega FB, Sjostrom M, Suni J, et al. Predictive validity of health-related fitness in youth: a systematic review. Brit J Sport Med. 2009;43(12):909-23.
140. Saltin B, Rowell LB. Functional Adaptations to Physical-Activity and Inactivity. Fed Proc. 1980;39(5):1506-13.
141. Hawley JA. Adaptations of skeletal muscle to prolonged, intense endurance training. Clin Exp Pharmacol P. 2002;29(3):218-22.
142. Hoppeler H, Fluck M. Plasticity of skeletal muscle mitochondria: Structure and function. Med Sci Sport Exer. 2003;35(1):95-104.

143. Prior BM, Lloyd PG, Yang HT, Terjung RL. Exercise-induced vascular remodeling. Exercise and Sport Sciences Reviews. 2003;31(1):26-33.
144. Holloszy JO. A forty-year memoir of research on the regulation of glucose transport into muscle. Am J Physiol Endocrinol Metab. 2003;284(3):E453-67.
145. Ivy JL, Zderic TW, Fogt DL. Prevention and treatment of non-insulin-dependent diabetes mellitus. Exerc Sport Sci Rev. 1999;27:1-35.
146. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, et al. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. New Engl J Med. 1996;335(18):1357-62.
147. Santos JM, Ribeiro SB, Gaya AR, Appell HJ, Duarte JA. Skeletal Muscle Pathways of Contraction-Enhanced Glucose Uptake. Int J Sports Med. 2008;29(10):785-94.
148. Wojtaszewski JFP, Nielsen JN, Richter EA. Exercise effects on muscle insulin signaling and action - Invited review: Effect of acute exercise on insulin signaling and action in humans. J Appl Physiol. 2002;93(1):384-92.
149. Mul JD, Stanford KI, Hirshman MF, Goodyear LJ. Exercise and Regulation of Carbohydrate Metabolism. Prog Mol Biol Transl. 2015;135:17-37.
150. Crouse SF, Obrien BC, Rohack JJ, Lowe RC, Green JS, Tolson H, et al. Changes in Serum-Lipids and Apolipoproteins after Exercise in Men with High Cholesterol - Influence of Intensity. J Appl Physiol. 1995;79(1):279-86.
151. Kiens B, Lithell H. Lipoprotein Metabolism Influenced by Training-Induced Changes in Human Skeletal-Muscle. J Clin Invest. 1989;83(2):558-64.
152. Dufaux B, Order U, Muller R, Hollmann W. Delayed-Effects of Prolonged Exercise on Serum-Lipoproteins. Metabolism-Clinical and Experimental. 1986;35(2):105-9.
153. Seip RL, Moulin P, Cocke T, Tall A, Kohrt WM, Mankowitz K, et al. Exercise Training Decreases Plasma Cholesteryl Ester Transfer Protein. Arterioscler Thromb. 1993;13(9):1359-67.
154. Taskinen MR, Nikkila EA. High-Density Lipoprotein Subfractions in Relation to Lipoprotein-Lipase Activity of Tissues in Man Evidence for Reciprocal Regulation of Hdl2 and Hdl3 Levels by Lipoprotein-Lipase. Clinica Chimica Acta. 1981;112(3):325-32.
155. Kiens B. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev. 2006;86(1):205-43.
156. Kelley GA, Kelley KS. Effects of exercise in the treatment of overweight and obese children and adolescents: a systematic review of meta-analyses. J Obes. 2013;2013:783103.
157. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circ Res. 2005;96(9):939-49.
158. Cayres SU, Agostinete RR, Antunes BDM, Lira FS, Fernandes RA. Impact of physical exercise/activity on vascular structure and inflammation in pediatric populations: A literature review. J Spec Pediatr Nurs. 2016;21(3):99-108.
159. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. C-reactive protein, interleukin-6 and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. Circulation. 2005;111(14):E190-E.
160. Balagopal P, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, et al. Nontraditional Risk Factors and Biomarkers for Cardiovascular Disease: Mechanistic, Research, and Clinical Considerations for Youth A Scientific Statement From the American Heart Association. Circulation. 2011;123(23):2749-69.
161. Alpert BS. Exercise as a therapy to control hypertension in children. Int J Sports Med. 2000;21:S94-S6.
162. Garcia-Hermoso A, Saavedra JM, Escalante Y. Effects of exercise on resting blood pressure in obese children: a meta-analysis of randomized controlled trials. Obesity Reviews. 2013;14(11):919-28.
163. Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA, et al. Exercise and hypertension. Med Sci Sport Exer. 2004;36(3):533-53.

164. Scherrer U, Sartori C. Defective nitric oxide synthesis: a link between metabolic insulin resistance, sympathetic overactivity and cardiovascular morbidity. Eur J Endocrinol. 2000;142(4):315-23.
165. Schutte NM, Nederend I, Hudziak JJ, Bartels M, de Geus EJC. Twin-sibling study and meta-analysis on the heritability of maximal oxygen consumption. Physiol Genomics. 2016;48(3):210-9.
166. Bouchard C, Pérusse L. Heredity, activity level, fitness, and health. In: Bouchard C, Shephard RJ, Stephens T, editors. Physical Activity, Fitness, and Health: International Proceedings and Consensus Statement: Champaign, IL: Human Kinetics; 1994. p. 106-18.
167. Story M, Nanney MS, Schwartz MB. Schools and obesity prevention: creating school environments and policies to promote healthy eating and physical activity. Milbank Q. 2009;87(1):71-100.
168. Pate RR, Davis MG, Robinson TN, Stone EJ, McKenzie TL, Young JC. Promoting physical activity in children and youth - A leadership role for schools - A scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism (Physical Activity Committee) in collaboration with the Councils on Cardiovascular Disease in the Young and Cardiovascular Nursing. Circulation. 2006;114(11):1214-24.
169. Dobbins M, Husson H, DeCorby K, LaRocca RL. School-based physical activity programs for promoting physical activity and fitness in children and adolescents aged 6 to 18. Cochrane Database Syst Rev. 2013;2:CD007651.
170. Kriemler S, Meyer U, Martin E, van Sluijs EM, Andersen LB, Martin BW. Effect of school-based interventions on physical activity and fitness in children and adolescents: a review of reviews and systematic update. Br J Sports Med. 2011;45(11):923-30.
171. Durlak JA, DuPre EP. Implementation matters: a review of research on the influence of implementation on program outcomes and the factors affecting implementation. Am J Community Psychol. 2008;41(3-4):327-50.
172. McKay HA, Macdonald HM, Nettlefold L, Masse LC, Day M, Naylor PJ. Action Schools! BC implementation: from efficacy to effectiveness to scale-up. Brit J Sport Med. 2015;49(4):210-U16.
173. Morgan PJ, Hansen V. Classroom Teachers' Perceptions of the Impact of Barriers to Teaching Physical Education on the Quality of Physical Education Programs. Res Q Exercise Sport. 2008;79(4):506-16.
174. Nathan N, Elton B, Babic M, McCarthy N, Sutherland R, Presseau J, et al. Barriers and facilitators to the implementation of physical activity policies in schools: A systematic review. Prev Med. 2018;107:45-53.
175. Sharma M. International school-based interventions for preventing obesity in children. Obesity Reviews. 2007;8(2):155-67.
176. Resaland GK, Moe VF, Aadland E, Steene-Johannessen J, Glosvik O, Andersen JR, et al. Active Smarter Kids (ASK): Rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on children's academic performance and risk factors for non-communicable diseases. BMC Public Health. 2015;15:709.
177. Steene-Johannessen J, Kolle E, Anderssen SA, Andersen LB. Cardiovascular disease risk factors in a population-based sample of Norwegian children and adolescents. Scand J Clin Lab Invest. 2009;69(3):380-6.
178. Riddoch C, Edwards D, Page A, Froberg K, Anderssen SA, Wedderkopp N, et al. The European Youth Heart Study—Cardiovascular Disease Risk Factorsin Children: Rationale, Aims, Study Design, and Validation of Methods. Journal of Physical Activity and Health. 2005(2):115-29.
179. Kristensen PL, Moeller NC, Korsholm L, Kolle E, Wedderkopp N, Froberg K, et al. The association between aerobic fitness and physical activity in children and adolescents: the European youth heart study. Eur J Appl Physiol. 2010;110(2):267-75.
180. Kriemler S, Zahner L, Schindler C, Meyer U, Hartmann T, Hebestreit H, et al. Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial. BMJ. 2010;340:c785.

181. Meyer U, Schindler C, Zahner L, Ernst D, Hebestreit H, van Mechelen W, et al. Long-Term Effect of a School-Based Physical Activity Program (KISS) on Fitness and Adiposity in Children: A Cluster-Randomized Controlled Trial. Plos One. 2014;9(2).
182. Bugge A, El-Naaman B, Dencker M, Froberg K, Holme IM, McMurray RG, et al. Effects of a three-year intervention: the Copenhagen School Child Intervention Study. Med Sci Sports Exerc. 2012;44(7):1310-7.
183. Zipf G, Chiappa M, Porter KS, Ostchega Y, Lewis BG, Dostal J. National health and nutrition examination survey: plan and operations, 1999-2010. Vital Health Stat 1. 2013(56):1-37.
184. Zahner L, Puder JJ, Roth R, Schmid M, Guldimann R, Puhse U, et al. A school-based physical activity program to improve health and fitness in children aged 6-13 years ("Kinder-Sportstudie KISS"): study design of a randomized controlled trial [ISRCTN15360785]. Bmc Public Health. 2006;6.
185. CDC. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. Questionnaires, Datasets, and Related Documentation; https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.
186. Steiner MJ, Skinner AC, Perrin EM. Fasting might not be necessary before lipid screening: a nationally representative cross-sectional study. Pediatrics. 2011;128(3):463-70.
187. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
188. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
189. Eiberg S, Hasselstrom H, Gronfeldt V, Froberg K, Cooper A, Andersen LB. Physical fitness as a predictor of cardiovascular disease risk factors in 6-to 7-year-old Danish children: The Copenhagen School-Child Intervention Study. Pediatric Exercise Science. 2005;17(2):161-70.
190. Pate RR, Wang CY, Dowda M, Farrell SW, O'Neill JR. Cardiorespiratory fitness levels among US youth 12 to 19 years of age: findings from the 1999-2002 National Health and Nutrition Examination Survey. Arch Pediatr Adolesc Med. 2006;160(10):1005-12.
191. Mamen A, Resaland GK, Mo DA, Andersen LB. Comparison of peak oxygen uptake in boys exercising on treadmill and cycle ergometers. Gazzetta Medica Italiana. 2008;167(1):15-21.
192. Esliger DW, Copeland JL, Barnes JD, Tremblay MS. Standardizing and Optimizing the Use of Accelerometer Data for Free-Living Physical Activity Monitoring. Journal of Physical Activity & Health. 2005;2(3):366-83.
193. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. J Sport Sci. 2008;26(14):1557-65.
194. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of Accelerometer Cut Points for Predicting Activity Intensity in Youth. Med Sci Sport Exer. 2011;43(7):1360-8.
195. White WB, Anwar YA. Evaluation of the overall efficacy of the Omron office digital blood pressure HEM-907 monitor in adults. Blood Press Monit. 2001;6(2):107-10.
196. El Assaad MA, Topouchian JA, Darne BM, Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002;7(4):237-41.
197. Aadland E, Andersen LB, Lerum O, Resaland GK. The Andersen aerobic fitness test: New peak oxygen consumption prediction equations in 10 and 16-year olds. Scand J Med Sci Sports. 2017.
198. Assoc WM. World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Jama-J Am Med Assoc. 2013;310(20):2191-4.
199. Stavnsbo M, Resaland GK, Anderssen SA, Steene-Johannessen J, Domazet SL, Skrede T, et al. Reference values for cardiometabolic risk scores in children and adolescents – suggesting a common standard. Atherosclerosis. 2018;278:299-306.
200. Little RJ, D'Agostino R, Cohen ML, Dickersin K, Emerson SS, Farrar JT, et al. The Prevention and Treatment of Missing Data in Clinical Trials. New Engl J Med. 2012;367(14):1355-60.

201. Resaland GK, Aadland E, Nilsen AKO, Bartholomew JB, Andersen LB, Anderssen SA. The effect of a two-year school-based daily physical activity intervention on a clustered CVD risk factor score-The Sogndal school-intervention study. Scand J Med Sci Sports. 2018;28(3):1027-35.
202. Resaland GK, Moe VF, Bartholomew JB, Andersen LB, McKay HA, Anderssen SA, et al. Gender-specific effects of physical activity on children's academic performance: The Active Smarter Kids cluster randomized controlled trial. Prev Med. 2018;106:171-6.
203. Ruiz JR, Cavero-Redondo I, Ortega FB, Welk GJ, Andersen LB, Martinez-Vizcaino V. Cardiorespiratory fitness cut points to avoid cardiovascular disease risk in children and adolescents; what level of fitness should raise a red flag? A systematic review and meta-analysis. Br J Sports Med. 2016.
204. Twisk JWR. Applied Multilevel Analysis. A Practical Guide. Cambridge, UK: Cambridge University Press; 2006.
205. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents,. Pediatrics. 2004;114(2 Suppl 4th Report):555-76.
206. Gurka MJ, Ice CL, Sun SS, Deboer MD. A confirmatory factor analysis of the metabolic syndrome in adolescents: an examination of sex and racial/ethnic differences. Cardiovasc Diabetol. 2012;11:128.
207. Goodman E, Daniels SR, Meigs JB, Dolan LM. Instability in the diagnosis of metabolic syndrome in adolescents. Circulation. 2007;115(17):2316-22.
208. Morrison JA, Laskarzewski PM, Rauh JL, Brookman R, Mellies M, Frazer M, et al. Lipids, lipoproteins, and sexual maturation during adolescence: the Princeton maturation study. Metabolism. 1979;28(6):641-9.
209. Moran A, Jacobs DR, Jr., Steinberger J, Steffen LM, Pankow JS, Hong CP, et al. Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. Circulation. 2008;117(18):2361-8.
210. Kelly AS, Steinberger J, Jacobs DR, Hong CP, Moran A, Sinaiko AR. Predicting cardiovascular risk in young adulthood from the metabolic syndrome, its component risk factors, and a cluster score in childhood. Int J Pediatr Obes. 2011;6(2-2):e283-9.
211. Moran A, Jacobs DR, Jr., Steinberger J, Hong CP, Prineas R, Luepker R, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes. 1999;48(10):2039-44.
212. Cook S, Auinger P, Huang TT. Growth curves for cardio-metabolic risk factors in children and adolescents. J Pediatr. 2009;155(3):S6 e15-26.
213. Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, Croft JB. State of disparities in cardiovascular health in the United States. Circulation. 2005;111(10):1233-41.
214. Andersen LB, Henckel P, Saltin B. Risk-Factors for Cardiovascular-Disease in 16-19-Year-Old Teenagers. Journal of Internal Medicine. 1989;225(3):157-63.
215. Wynder EL, Williams CL, Laakso K, Levenstein M. Screening for risk factors for chronic disease in children from fifteen countries. Prev Med. 1981;10(2):121-32.
216. Beevers G, Lip GY, O'Brien E. ABC of hypertension. Blood pressure measurement. Part I-sphygmomanometry: factors common to all techniques. BMJ. 2001;322(7292):981-5.
217. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med. 1997;336(16):1117-24.
218. Jones DW, Appel LJ, Sheps SG, Roccella EJ, Lenfant C. Measuring blood pressure accurately: new and persistent challenges. JAMA. 2003;289(8):1027-30.
219. Duncombe SL, Voss C, Harris KC. Oscillometric and auscultatory blood pressure measurement methods in children: a systematic review and meta-analysis. J Hypertens. 2017;35(2):213-24.
220. Gungor N, Saad R, Janosky J, Arslanian S. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr-Us. 2004;144(1):47-55.
221. Andersen LB, Boreham CA, Young IS, Davey Smith G, Gallagher AM, Murray L, et al. Insulin sensitivity and clustering of coronary heart disease risk factors in young adults. The Northern Ireland Young Hearts Study. Prev Med. 2006;42(1):73-7.

222. Bertrais S, Balkau B, Charles MA, Vol S, Calvet C, Tichet J, et al. Puberty-associated differences in total cholesterol and triglyceride levels according to sex in French children aged 10-13 years. Ann Epidemiol. 2000;10(5):316-23.
223. Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. Horm Res Paediatr. 2012;77(3):137-45.
224. Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. Pediatrics. 2009;123(5):e932-9.
225. Assmann G, Gotto AM, Jr. HDL cholesterol and protective factors in atherosclerosis. Circulation. 2004;109(23 Suppl 1):III8-14.
226. Kwiterovich PO, Vining EPG, Pyzik P, Skolasky R, Freeman JM. Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children. Jama-J Am Med Assoc. 2003;290(7):912-20.
227. Dron JS, Hegele RA. Genetics of Triglycerides and the Risk of Atherosclerosis. Curr Atheroscler Rep. 2017;19(7):31.
228. WHO. Adolescent obesity and related behaviours: trends and inequalities in the WHO European Region, 2002–2014. Observations from the Health Behaviour in School-aged Children (HBSC) WHO collaborative cross-national study. http://www.euro.who.int/__data/assets/pdf_file/0019/339211/WHO_ObesityReport_2017_v3.pdf; 2017.
229. Skinner AC, Ravanbakht SN, Skelton JA, Perrin EM, Armstrong SC. Prevalence of Obesity and Severe Obesity in US Children, 1999-2016. Pediatrics. 2018.
230. Resaland GK, Aadland E, Moe VF, Aadland KN, Skrede T, Stavnsbo M, et al. Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. Prev Med. 2016;91:322-8.
231. Garnett SP, Baur LA, Srinivasan S, Lee JW, Cowell CT. Body mass index and waist circumference in midchildhood and adverse cardiovascular disease risk clustering in adolescence. American Journal of Clinical Nutrition. 2007;86(3):549-55.
232. Andersen LB, Bugge A, Dencker M, Eiberg S, El-Naaman B. The association between physical activity, physical fitness and development of metabolic disorders. Int J Pediatr Obes. 2011;6 Suppl 1:29-34.
233. Bailey DP, Boddy LM, Savory LA, Denton SJ, Kerr CJ. Associations between cardiorespiratory fitness, physical activity and clustered cardiometabolic risk in children and adolescents: the HAPPY study. Eur J Pediatr. 2012;171(9):1317-23.
234. Katzmarzyk PT, Church TS, Blair SN. Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men. Arch Intern Med. 2004;164(10):1092-7.
235. Waters L, Reeves M, Fjeldsoe B, Eakin E. Control group improvements in physical activity intervention trials and possible explanatory factors: a systematic review. J Phys Act Health. 2012;9(6):884-95.
236. Health TNDo. Physical activity in Norwegian 6, 9 and 15-year olds.
237. Stavnsbo M, Skrede T, Aadland E, Aadland KN, Chinapaw M, Anderssen SA, et al. Cardiometabolic risk factor levels in Norwegian children compared to international reference values: The ASK study. Unpublished results - *In review*. 2019.
238. Sun C, Pezic A, Tikellis G, Ponsonby AL, Wake M, Carlin JB, et al. Effects of school-based interventions for direct delivery of physical activity on fitness and cardiometabolic markers in children and adolescents: a systematic review of randomized controlled trials. Obes Rev. 2013.
239. Pozuelo-Carrascosa DP, Garcia-Hermoso A, Alvarez-Bueno C, Sanchez-Lopez M, Martinez-Vizcaino V. Effectiveness of school-based physical activity programmes on cardiorespiratory fitness in children: a meta-analysis of randomised controlled trials. Br J Sports Med. 2017.

240. Resaland GK, Andersen LB, Mamen A, Anderssen SA. Effects of a 2-year school-based daily physical activity intervention on cardiorespiratory fitness: the Sogndal school-intervention study. Scand J Med Sci Sports. 2011;21(2):302-9.
241. WHO. Water, Sanitation and Health Team. Mercury in health care : policy paper. Geneva, World Health Organization. http://www.who.int/iris/handle/10665/69129; 2005.
242. Ogedegbe G, Pickering T. Principles and techniques of blood pressure measurement. Cardiol Clin. 2010;28(4):571-86.
243. O'Brien E PJ, Littler W, de Swiet M, Padfield PL, Altman D, Bland M, Coats A, Atkins N. The British Hypertension Society protocol for the evaluation of blood pressure measuring devices. J Hypertens. 1993;11 (suppl 2):43-62.
244. O'Brien E, Atkins N, Stergiou G, Karpettas N, Parati G, Asmar R, et al. European Society of Hypertension International Protocol revision 2010 for the validation of blood pressure measuring devices in adults. Blood Press Monit. 2010;15(1):23-38.
245. Instrumentation AftAoM. Non-invasive sphygmomanometers - part 2: clinical validation of automated measurement type. ANSI/AAMI/ISO 81060–2. Association for the Advancement of Medical Instrumentatio. 2013.
246. Tolonen H, Koponen P, Naska A, Mannisto S, Broda G, Palosaari T, et al. Challenges in standardization of blood pressure measurement at the population level. BMC Med Res Methodol. 2015;15:33.
247. Kawahara J, Sano H, Fukuzaki H, Saito K, Hirouchi H. Acute effects of exposure to cold on blood pressure, platelet function and sympathetic nervous activity in humans. Am J Hypertens. 1989;2(9):724-6.
248. Campbell NR, McKay DW, Chockalingam A, Fodor JG. Errors in assessment of blood pressure: patient factors. Can J Public Health. 1994;85 Suppl 2:S12-7.
249. Bergman RN, Stefanovski D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, et al. A Better Index of Body Adiposity. Obesity. 2011;19(5):1083-9.
250. Boeke CE, Oken E, Kleinman KP, Rifas-Shiman SL, Taveras EM, Gillman MW. Correlations among adiposity measures in school-aged children. BMC Pediatr. 2013;13:99.
251. Ross R, Berentzen T, Bradshaw AJ, Janssen I, Kahn HS, Katzmarzyk PT, et al. Diagnostic in obesity comorbidities - Does the relationship between waist circumference, morbidity and mortality depend on measurement protocol for waist circumference? Obesity Reviews. 2008;9(4):312-25.
252. WHO. WHO STEPwise approach to surveillance (STEPS). Geneva, World Health Organization (WHO). 2008.
253. NHLBI. Obesity Education Initiative. The practical guide: Identification, evaluation and treatment of overweight and obesity in adults. National Institutes of Health. 2000;(NIH Publication Number 00-4084).
254. Harrington DM, Staiano AE, Broyles ST, Gupta AK, Katzmarzyk PT. Waist circumference measurement site does not affect relationships with visceral adiposity and cardiometabolic risk factors in children. Pediatr Obes. 2013;8(3):199-206.
255. WHO. Waist Circumference and Waist-Hip Ratio. Report of a WHO Expert Consultation. Geneva, World Health Organization. 2008.
256. WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO expert consultation. Geneva, World Health Organization (WHO). 1995.
257. Yorkin M, Spaccarotella K, Martin-Biggers J, Quick V, Byrd-Bredbenner C. Accuracy and consistency of weights provided by home bathroom scales. BMC Public Health. 2013;13:1194.
258. Ulijaszek SJ, Kerr DA. Anthropometric measurement error and the assessment of nutritional status. Br J Nutr. 1999;82(3):165-77.
259. Kispert CP, Merrifield HH. Interrater reliability of skinfold fat measurements. Phys Ther. 1987;67(6):917-20.
260. Ayling RM. Chapter 10 - Chemistry biochemistry of nutrition. Churchil Livingstone2014.

261. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation. 2008;118(20):2047-56.
262. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation. 2008;118(10):993-1001.
263. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting Is Not Routinely Required for Determination of a Lipid Profile: Clinical and Laboratory Implications Including Flagging at Desirable Concentration Cutpoints - A Joint Consensus Statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Acta Bioquim Clin L. 2016;50(3):469-87.
264. Grundy SM, Becker D, Clark LT, Cooper RS, Denke MA, Howard WJ, et al. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation. 2002;106(25):3143-421.
265. Baskurt OK, Yalcin O, Gungor F, Meiselman HJ. Hemorheological parameters as determinants of myocardial tissue hematocrit values. Clin Hemorheol Micro. 2006;35(1-2):45-50.
266. Kupke IR, Zeugner S, Gottschalk A, Kather B. Differences in lipid and lipoprotein concentrations of capillary and venous blood samples. Clin Chim Acta. 1979;97(2-3):279-83.
267. Kafonek SD, Donovan L, Lovejoy KL, Bachorik PS. Biological variation of lipids and lipoproteins in fingerstick blood. Clin Chem. 1996;42(12):2002-7.
268. Sedgwick MJ, Morris JG, Nevill ME, Barrett LA. Comparison of lipid and lipoprotein concentrations obtained when using capillary and venous plasma. Atherosclerosis. 2011;218(2):E10-E.
269. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237(3):E214-23.
270. Steffes MW, Sacks DB. Measurement of circulating glucose concentrations: The time is now for consistency among methods and types of samples. Clin Chem. 2005;51(9):1569-70.
271. Kang JG, Park CY, Ihm SH, Park SW. A Potential Issue with Screening Prediabetes or Diabetes Using Serum Glucose: A Delay in Diagnosis. Diabetes Metab J. 2016;40(5):414-7.
272. Larssoncohn U. Differences between Capillary and Venous-Blood Glucose during Oral Glucose-Tolerance Tests. Scand J Clin Lab Inv. 1976;36(8):805-8.
273. LeMura LM, von Duvillard SP, Cohen SL, Root CJ, Chelland SA, Andreacci J, et al. Treadmill and cycle ergometry testing in 5- to 6-year-old children. Eur J Appl Physiol. 2001;85(5):472-8.
274. Tomkinson GR, Leger LA, Olds TS, Cazorla G. Secular trends in the performance of children and adolescents (1980-2000): an analysis of 55 studies of the 20m shuttle run test in 11 countries. Sports Med. 2003;33(4):285-300.
275. Tomkinson GR, Lang JJ, Tremblay MS, Dale M, LeBlanc AG, Belanger K, et al. International normative 20 m shuttle run values from 1 142 026 children and youth representing 50 countries. Brit J Sport Med. 2017;51(21):1545-+.
276. Ruiz JR, Silva G, Oliveira N, Ribeiro JC, Oliveira JF, Mota J. Criterion-related validity of the 20-m shuttle run test in youths aged 13-19 years. J Sport Sci. 2009;27(9):899-906.
277. CDC. Center for Disease Control and Prevention. http://www.cdc.gov/nchs/tutorials/Nhanes/index_current.htm [
278. Finn K, Kaj M, Marton O, Karsai I, Saint-Maurice P, Csányi T. Accuracy Of The NHANES Treadmill Test In Estimating Aerobic Fitness In Adolescents. Medicine & Science in Sports & Exercise. 2015;47:298-undefined.
279. Knaier R, Infanger D, Niemeyer M, Cajochen C, Schmidt-Trucksass A. In Athletes, the Diurnal Variations in Maximum Oxygen Uptake Are More Than Twice as Large as the Day-to-Day Variations. Front Physiol. 2019;10.
280. Morgan DW, Bransford DR, Costill DL, Daniels JT, Howley ET, Krahenbuhl GS. Variation in the Aerobic Demand of Running among Trained and Untrained Subjects. Med Sci Sport Exer. 1995;27(3):404-9.

281. Lussier L, Buskirk ER. Effects of an endurance training regimen on assessment of work capacity in prepubertal children. Ann N Y Acad Sci. 1977;301:734-47.
282. Peronnet F, Thibault G. Mathematical-Analysis of Running Performance and World Running Records. J Appl Physiol. 1989;67(1):453-65.
283. Cureton KJ, Boileau RA, Lohman TG, Misner JE. Determinants of distance running performance in children: analysis of a path model. Res Q. 1977;48(2):270-9.
284. McCormick A, Meijen C, Marcora S. Psychological Determinants of Whole-Body Endurance Performance. Sports Med. 2015;45(7):997-1015.
285. Steele RM, Brage S, Corder K, Wareham NJ, Ekelund U. Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. J Appl Physiol (1985). 2008;105(1):342-51.
286. Wannamethee SG, Shaper AG, Lennon L, Morris RW. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. Arch Intern Med. 2005;165(22):2644-50.
287. Golden SH, Folsom AR, Coresh J, Sharrett AR, Szklo M, Brancati F. Risk factor groupings related to insulin resistance and their synergistic effects on subclinical atherosclerosis - The Atherosclerosis Risk in Communities Study. Diabetes. 2002;51(10):3069-76.
288. Friend A, Craig L, Turner S. The prevalence of metabolic syndrome in children: a systematic review of the literature. Metab Syndr Relat Disord. 2013;11(2):71-80.
289. Gustafson JK, Yanoff LB, Easter BD, Brady SM, Keil MF, Roberts MD, et al. The Stability of Metabolic Syndrome in Children and Adolescents. J Clin Endocr Metab. 2009;94(12):4828-34.
290. Srinivasan SR, Myers L, Berenson GS. Predictability of childhood adiposity and insulin for developing insulin resistance syndrome (syndrome X) in young adulthood - The Bogalusa Heart Study. Diabetes. 2002;51(1):204-9.
291. Kollias A, Dafni M, Poulidakis E, Ntineri A, Stergiou GS. Out-of-office blood pressure and target organ damage in children and adolescents: a systematic review and meta-analysis. J Hypertens. 2014;32(12):2315-31; discussion 31.
292. Mourad JJ. The evolution of systolic blood pressure as a strong predictor of cardiovascular risk and the effectiveness of fixed-dose ARB/CCB combinations in lowering levels of this preferential target. Vasc Health Risk Manag. 2008;4(6):1315-25.
293. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. Lancet. 2016;387(10022):957-67.
294. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444(7121):875-80.
295. Wang YF, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. American Journal of Clinical Nutrition. 2005;81(3):555-63.
296. Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J. Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk--a review of the literature. Eur J Clin Nutr. 2010;64(1):16-22.
297. Maynard LM, Wisemandle W, Roche AF, Chumlea WC, Guo SS, Siervogel RM. Childhood body composition in relation to body mass index. Pediatrics. 2001;107(2):344-50.
298. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of Type 1 and Type 2 Diabetes Among Children and Adolescents From 2001 to 2009. Jama-J Am Med Assoc. 2014;311(17):1778-86.
299. Prospective Studies C, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet. 2007;370(9602):1829-39.
300. Priestley M, Biesta G, Robinson S. Teacher agency: An ecological approach. Bloomsbury Publishing. 2015.

301. Lerum Ø, Bartholomew JB, McKay H, Resaland GK, Tjomsland HE, Anderssen SA, et al. Primary school teachers perceptions and maintenance of a school-based physical activity intervention. In review. 2019.
302. Finn KE, McInnis KJ. Teachers' and students' perceptions of the active science curriculum: Incorporating physical activity into middle school science classrooms. Physical Educator. 2014;71(2):234.
303. Howie EK, Newman-Norlund RD, Pate RR. Smiles count but minutes matter: responses to classroom exercise breaks. Am J Health Behav. 2014;38(5):681-9.
304. Martin R, Murtagh EM. Preliminary findings of Active Classrooms: An intervention to increase physical activity levels of primary school children during class time. Teach Teach Educ. 2015;52:113-27.
305. Stylianou M, Kulinna PH, Naiman T. '...because there's nobody who can just sit that long': Teacher perceptions of classroom-based physical activity and related management issues. Eur Phys Educ Rev. 2016;22(3):390-408.
306. White RT, Arzi HJ. Longitudinal studies: Designs, validity, practicality, and value. Res Sci Educ. 2005;35(1):137-49.
307. Malina R, Bouchard C, Bar-Or O. Growth, Maturation, and Physical Activity. 2nd ed Champaign (EUA): Human Kinetics. 2004.
308. Santos R, Moreira C, Abreu S, Lopes L, Ruiz JR, Moreira P, et al. Parental Education Level Is Associated With Clustering of Metabolic Risk Factors in Adolescents Independently of Cardiorespiratory Fitness, Adherence to the Mediterranean Diet, or Pubertal Stage. Pediatr Cardiol. 2014;35(6):959-64.
309. Fahlman MM, Hall HL, Lock R. Ethnic and socioeconomic comparisons of fitness, activity levels, and barriers to exercise in high school females. J School Health. 2006;76(1):12-7.
310. Bohr AD, Brown DD, Laurson KR, Smith PJK, Bass RW. Relationship Between Socioeconomic Status and Physical Fitness in Junior High School Students. J School Health. 2013;83(8):542-7.
311. Coleman L, Coleman J. The measurement of puberty: a review. J Adolesc. 2002;25(5):535-50.
312. Desmangles JC, Lappe JM, Lipaczewski G, Haynatzki G. Accuracy of pubertal Tanner staging self-reporting. J Pediatr Endocrinol Metab. 2006;19(3):213-21.
313. Carskadon MA, Harvey K, Duke P, Anders TF, Litt IF, Dement WC. Pubertal changes in daytime sleepiness. Sleep. 1980;2(4):453-60.
314. Norris SA, Richter LM. Usefulness and reliability of tanner pubertal self-rating to urban Black adolescents in South Africa. J Res Adolescence. 2005;15(4):609-24.
315. Boas SR, Falsetti D, Murphy TD, Orenstein DM. Validity of self-assessment of sexual maturation in adolescent male patients with cystic fibrosis. J Adolesc Health. 1995;17(1):42-5.
316. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135-43.
317. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320(7244):1240-3.

PAPER I

Published: Atherosclerosis

Accepted: October 2018

Atherosclerosis 278 (2018) 299–306

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Reference values for cardiometabolic risk scores in children and adolescents: Suggesting a common standard

Mette Stavnsbo[a,b,*], Geir K. Resaland[a], Sigmund A. Anderssen[a,b], Jostein Steene-Johannessen[b,c], Sidsel L. Domazet[d], Turid Skrede[a,b], Luis B. Sardinha[e], Susi Kriemler[f], Ulf Ekelund[b], Lars B. Andersen[a,b], Eivind Aadland[a]

[a] Western Norway University of Applied Sciences, Department of Sport, Food and Natural Sciences, PO BOX 133, N-6856, Sogndal, Norway
[b] Norwegian School of Sport Sciences, Department of Sports Medicine, PO Box 4014, Sognsveien 220, Ullevaal Stadion, Oslo, Norway
[c] Kristiania University College, Faculty of Health Sciences, PO Box 1155, Sentrum, 0107, Oslo, Norway
[d] University of Southern Denmark, Department of Sports Science and Clinical Biomechanics, Center of Research in Childhood Health, Campusvej 55, 5230, Odense M, Denmark
[e] Universidade de Lisboa, Fac Motricidade Humana, CIPER, Exercise and Health Laboratory, Estrada Dacosth, Cruz-Quebrada, 1499, Lisbon, Portugal
[f] University of Zürich, Epidemiology, Biostatistics and Prevention Institute, Hirschengraben 84, 8001, Zürich, Switzerland

HIGHLIGHTS

- Reference values for 14 cardiometabolic risk factors in 6–18 year olds are provided.
- Based on 22,479 observations from European and American studies.
- Easy to apply to standardize cardiometabolic risk factors.
- No similar reference material is available.
- Make comparability of continuous clustered risk scores between populations possible.

ARTICLE INFO

Keywords:
Cardiovascular disease risk factors
Metabolic syndrome
Reference values
Standardization
Pediatric

ABSTRACT

Background and aims: International reference values for cardiometabolic risk variables, to allow for standardization of continuous risk scores in children, are not currently available. The aim of this study was to provide international age- and gender-specific reference values for cardiometabolic risk factors in children and adolescents.

Methods: Cohorts of children sampled from different parts of Europe (North, South, Mid and Eastern) and from the United States were pooled. In total, 22,479 observations (48.7% European *vs.* 51.3% American), 11,234 from girls and 11,245 from boys, aged 6–18 years were included in the study. Linear mixed-model regression analysis was used to analyze the associations between age and each cardiometabolic risk factor.

Results: Reference values for 14 of the most commonly used cardiometabolic risk variables in clustered risk scores were calculated and presented by age and gender: systolic blood pressure (SBP), diastolic blood pressure (DBP), waist circumference (WC), body mass index (BMI), sum of 4 skinfolds (sum4skin), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), TC:HDL-C ratio, glucose, insulin, homeostatic model assessment-score (HOMA-score), and cardiorespiratory fitness (CRF).

Conclusions: This study suggests a common standard to define cardiometabolic risk in children. Adapting this approach makes single risk factors and clustered cardiometabolic disease risk scores comparable to the reference material itself and comparable to cardiometabolic risk values in studies using the same strategy. This unified approach therefore increases the prospect to estimate and compare prevalence and trends of cardiometabolic risk in children when using continuous cardiometabolic risk scores.

* Corresponding author. Western Norway University of Applied Sciences, Department of Sport, Food and Natural Sciences, PO Box 133, N-6856, Sogndal, Norway.
E-mail address: Mette.Stavnsbo@hvl.no (M. Stavnsbo).

https://doi.org/10.1016/j.atherosclerosis.2018.10.003
Received 26 July 2018; Received in revised form 12 September 2018; Accepted 4 October 2018
Available online 06 October 2018

1. Introduction

For almost a century, epidemiologists and other healthcare professionals have been aware of the biological phenomenon that risk factors for cardiovascular diseases (CVD) cluster in some individuals [1]. However, it was not until Reaven's Banting Medal award lecture in 1988 that the pathophysiological condition “Syndrome X” [2], which grounded the later “metabolic syndrome” (MetS), was unified and gained considerable foothold in the medical literature.

The interest in the MetS has steadily increased over the years [3], however, despite efforts of harmonizing the syndrome [4], limited consensus exists regarding its components and use. A number of definitions of the syndrome have been proposed, of which the most used are the definitions suggested by the World Health Organization (WHO) [5], National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) [6], and International Diabetes Federation (IDF) [7]. Adjusted versions of these definitions have also been applied in children and adolescents (hereafter referred to only as *children*) [8–11]. Divergent definitions of the MetS do not only make comparison between studies and populations difficult in general, but the arbitrarily adjusted versions also pose a major limitation when estimating prevalence and trends of cardiometabolic risk in children.

Despite evidence associating the MetS to increased risk of developing CVD, using the commonly applied definitions [12,13], the use of dichotomized variables to create a clustered risk score ignores the continuous nature of risk and substantially decreases the available information and power of statistical analysis [14–16]. In addition, children have not yet established CVDs, which further makes the argument to use thresholds in children inadequate. Furthermore, definitions of the MetS derived from dichotomized risk variables often identify a relatively low prevalence of the MetS in pediatric populations (< 4%) [17]. Thus, the use of a continuous cluster risk score in children is arguably superior to a dichotomized score to overcome the limitations when adapting adult definitions to the pediatric population [3,18]. However, standardized reference values for cardiometabolic risk variables in children are not currently available. Such standards are needed to identify prevalence and trends of cardiometabolic risk in children and simultaneously aid comparability among studies.

Andersen and colleagues [19] published reference values for single cardiometabolic risk variables of adiposity, impaired glucose intolerance, dyslipidemia, hypertension, markers of inflammation, and cardiorespiratory fitness. The study pooled data from European and US cohorts, including 15 794 children between 6 and 18 years old, aiming to provide a basis for establishing the level of cardiometabolic risk in children. However, the study did not include log-transformed values for non-normally distributed variables, nor were age- and gender-specific variability estimates presented. Such values are required to allow for future standardization of cardiometabolic risk factors. Further, the authors assumed linearity of all risk factors across the 12-year age span, thus ignoring possible non-linear trends caused by biological maturation [20,21]. Hence, the purpose of the present study is to provide reference values for the most commonly used cardiometabolic risk factors in European and US children, while taking these previous limitations into account.

2. Materials and methods

2.1. Sample and study design

Cross-sectional data from 23 cohorts of children aged 6–18 years were pooled, as described earlier by Andersen and colleagues [19]. Data were divided into eight new cohorts for this study, representing data from the following countries: Denmark (European Youth Heart Study (EYHS 1997–1998 and 2003–2004) and Copenhagen School Child Intervention Study (CoSCIS 2001, 2004, and 2008)), Estonia (EYHS 1999), Norway (EYHS (1999–2000) and Physical Activity among Norwegian Children Study (PANCS) 2005–2006), Portugal (EYHS 1999–2000 and 2008), Switzerland (Kinder Sports Studie (KISS) 2005–2006 and 2009), and the United States (US National Health and Nutrition Examination Survey (NHANES) 2001–2002, 2003–2004, 2005–2006 and 2007–2008).

The majority of the European data (EYHS, KISS, and PANCS) were based on randomly selected samples, using public schools as the primary sampling unit. In PANCS, Statistics Norway randomly selected schools from all regions of the country that included 96% of all Norwegian children in the 4th and 10th grades in the sampling frame. Of those, 82% (n = 2299) participated and n = 2266 were included in the present study [22]. In EYHS, a two-stage cluster sampling procedure was used to recruit a minimum of 1000 girls and boys (9 and 15 years old) from each study location, randomly sampled from the schools' register lists. Schools were stratified according to the socio-economic character of the local area (urban or rural) and weighted according to size [23]. The overall participation rate was 74% in EYHS I (n = 4169), whereof this study included children from Estonia (n = 1174) and Norway (n = 754) [23]. We also included data from Danish (n = 1861) [24] and Portuguese (n = 1771) children from both EYHS I and EYHS II. The KISS physical activity (PA) intervention study represents data from two provinces of Switzerland, where 28 classes were randomly chosen from a sample of 190 consenting classes. Of the initial 502 included children at baseline (age 7 and 11 years), 96% were re-tested at the post-intervention tests [25] and 60% at the 3-year follow-up [26]. We excluded follow-up values from KISS for variables where a significant intervention effect were revealed. In total, 1309 observations were included from the KISS study in the present study. The cohort in CoSCIS PA intervention study consisted of representative Danish children from two suburbs of Copenhagen. In total, 696 children (68% of invited) participated at baseline from 46 preschool classes (aged 6–7 years), 613 of these were included in the post-intervention tests, and 513 in the 7-year follow-up [27]. We included all values from CoSCIS as no intervention effects were revealed between groups (n = 1812 observations). NHANES randomly selected representative North American children following a multi-stage selection procedure, using mostly single counties as the primary sample unit with probability proportionate to the measure of county size [28]. Compared to Andersen et al. [19] we extended the NHANES data to cover the same time period as the European data cover and to match the number of European and American children. Overall, n = 11 532 children from NHANES were included in the present study from biannual survey releases covering 7 years (2001–2008). In total, 22 479 valid observations (48.7% European *vs.* 51.3% American), 11 234 from girls and 11 245 from boys, in at least one of the 14 cardiometabolic risk variables of interest, were included.

All individual studies have been ethically approved prior to commencement of investigation and all participants and/or legal guardians provided informed consent. Details of the earlier pooling of cohorts, sampling procedures, data collections, analyses, and ethical approvals for the separate studies are described elsewhere [19,22,23,27–30].

2.2. Cardiometabolic risk factors

The variables included in the present study were systolic blood pressure (SBP), diastolic blood pressure (DBP), waist circumference (WC), body mass index (BMI), sum of four skinfolds (Sum4Skin), triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), the ratio of TC to HDL-C (TC:HDL-C ratio), glucose, insulin, homeostatic model assessment (HOMA) score and cardiorespiratory fitness (CRF).

2.2.1. Blood pressure

Similar for the studies included, SBP and DBP were measured when children were in a sitting position after at least 5 min of rest, at the mid-upper arm with an appropriately sized cuff. The European studies all

used oscillometric blood pressure devices whereas NHANES used the auscultation method by the mercury sphygmonometer. The mean of three measures was used in the analyses (if more than three measures were obtained, the mean of the last three was used).

2.2.2. Anthropometrics

WC was measured with an anthropometric tape around the abdomen at the end of a light respiration, either a) at the level of the umbilicus, or b) midway between the lower rib margin and the iliac crest, or c) at the smallest circumference between the ribcage and the iliac crest and half-way between the lower cost rim and the Spina iliaca anterior superior. WC was measured ones in all studies except EYHS that obtained two measures of WC and the mean values of those was used in the analysis. Height was measured without shoes to the nearest ≤ 0.5 cm and body weight was measured with an accuracy of ≤ 0.5 kg. BMI (kg/m^2) was calculated as weight (kg) divided by height squared (m^2). Skinfold thickness was measured with a Harpenden caliper at biceps, triceps, subscapular, and suprailiac sites and summed for the use in analyses.

2.2.3. Blood samples

Blood samples were collected during the morning hours in fasting children and stored at -70 to -80 °C until analysis. All studies draw intravenous blood samples from participants, except the Norwegian part of the EYHS that used capillary blood as the basis for the biochemical analyses. Children under the age of 12 years were not instructed to fast in NHANES, and non-fasting TC, HDL-C, and TG values were therefore corrected for fasting time (hours) according to Steiner et al. [31]. TC, HDL-C, and TG were measured by enzymatic methods, and we estimated LDL-C from TC, HDL-C and TG using the Friedewald formula [32]. Glucose was analyzed using the hexokinase method in all studies except CoSCIS that used the dehydrogenase methodology. Insulin was measured using an enzyme-linked immunosorbent assay. No values on glucose or insulin were obtained for children < 12 years in NHANES. HOMA score was defined as [insulin (pmol/L) * glucose (mmol/L)]/22.5 [33].

2.2.4. Cardiorespiratory fitness

Cardiorespiratory fitness (mL/kg/min) was assessed by either: a) directly measured VO_{2peak} from a graded maximal treadmill running test [34], or b) directly measured VO_{2peak} during a graded maximal cycle ergometer test [22], or c) a standardized submaximal treadmill running test [35], or d) a 20 m graded maximal multistage shuttle run field test [36]. All results obtained from cycle ergometer tests were multiplied by 1.05 to be comparable with results obtained by the directly measured VO_{2peak} treadmill running test [37]. NHANES did not measure CRF in children < 12 years. For more detailed test descriptions, please see the original studies [19,22,23,27,29,30].

2.3. Statistical analysis

Prior to performing the analyses, we assessed standardized residuals for all risk factors regressed by gender and age, and removed values with residuals ≥ 5 standard deviations (SD) from the mean (n = 261 values). WC, BMI, Sum4Skin, TG, TC:HDL-C ratio, insulin and HOMA score were thereafter logarithmically transformed (natural log) because of skewness.

Linear mixed-model regression analysis was used to analyze the associations between age and single risk factors, including the random intercept of cohort. The trend for age was tested in all models by evaluating first, second, and third-order terms of age (higher order terms were omitted to avoid overfitting). The highest order terms were retained in the models if statistically significant. Thus, reference values for variables where a linear trend for age was evident were calculated by the following regression equation: risk factor$_{ref_linear}$ = α + (β * age). Furthermore, reference values for variables showing a quadratic or cubic relationship with age were calculated by including a second-order term (risk factor$_{ref_quadratic}$ = α + (β_1 * age) + (β_2 * age^2)) or a second- and third-order term (risk factor$_{ref_cubic}$ = α + (β_1* age) + (β_2 * age^2) + (β_3 * age^3)), respectively. All analyses were conducted separately in boys and girls after verifying statistically significant interactions by gender (age*gender) for most variables. Age- and gender-specific reference values are presented as means and SD, where SDs were calculated as the mean of residuals from the regression models. All variables and regression equations are presented by gender and age as absolute values. Non-normally distributed variables are also presented as log-transformed values.

In addition, we performed a subgroup analysis using a broad selection of previously applied continuous clustered risk scores in the pediatric population [18,38,39] to investigate the comparability of differently constructed composite cardiometabolic risk scores using the Pearson correlation coefficients. Single risk variables were converted to z-scores, summarized, and divided by the total number of included variables to create comparable mean values for the clustered z-scores. Calculations were derived from the reference values suggested herein, representing children with valid data in all 14 risk variables (n = 6471).

An alpha-level of $p \leq 0.05$ was considered statistically significant. All statistical analyses were conducted using IBM SPSS version 23 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA).

3. Results

The reference material was primarily derived from cross-sectional data and baseline values (91% of the data) whereas a minor part (9% of the data) constitutes of longitudinal data from intervention studies without significant intervention effects. The contribution of data from each cohort and number of observations per single risk factor by age and gender are presented in Supplementary Table A and B, respectively.

Reference values are presented in Table 1 (absolute values) and Table 2 (log-transformed values for skewed variables). All variables showed statistically significant age-related associations in both genders. In general, a similar pattern in the cardiometabolic risk values with increased age was observed in both genders for the following variables: SBP, DBP, WC, BMI, LDL-C, glucose, insulin and HOMA score. Levels of SBP, DBP, WC, and BMI increased as the children aged (SBP flattening in girls from 11 years), whereas LDL-C decreased until the age of 15 years and increased minimally after 16 years. Until the age of approximately 13, levels of glucose increased in both genders and thereafter slightly decreased. Insulin and HOMA score levels both increased until the age of 15 years, thereafter curves reversed.

In girls, skinfold thickness increased linearly with age. TC and HDL-C increased until the age of eight, decreased slightly between the ages of nine and 15 years, and thereafter increased again. The TC:HDL-C ratio decreased linearly by age, although minimally. Levels of TG increased until the age of 12 years, plateaued, and then decreased from the age of 16 years. CRF levels increased until the girls were nine years of age and thereafter declined throughout the age span.

In boys, skinfold thickness increased with age, however, not to the same extent as in girls, and decreased after the age of 14 years. TC decreased linearly across ages, whereas HDL-C increased until the age of nine, decreased until 17 years of age, and thereafter increased slightly again. TG and TC:HDL-C ratio (from the age of seven) both increased with age. CRF also increased steadily as the boys aged. All gender- and age-specific reference values are calculated from the regression equations shown in Table 3. For a graphic illustration, please see the Supplementary material (Figure A-N).

The correlation coefficients between different clustered cardiometabolic risk scores, based on the derived reference values, are presented in Table 4. A moderate-to-strong relationship was found between all investigated cluster scores (r = [0.52–0.98], $p \leq 0.001$). The clustered cardiometabolic risk score, including DBP, TC, and HDL-C levels,

Table 1
Reference values for single risk factors by age and gender (*absolute values*).

	Age (years)	SBP (mmHg)	DBP (mmHg)	WC (cm)	BMI (kg/m^2)	Sum4Skin (mm)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TC:HDL-C ratio	TG (mmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	HOMA score	CRF (mL/kg/min)
						Girls									
Mean	6	94.6	56.8	48.1	14.4	30.7	4.29	1.48	2.58	3.00	0.69	4.38	25.5	0.82	41.1
	7	96.5	57.9	52.4	15.3	32.7	4.37	1.51	2.57	3.00	0.72	4.61	28.5	0.99	42.2
	8	98.4	59.0	56.1	16.2	34.8	4.40	1.53	2.54	2.99	0.75	4.78	33.5	1.21	42.8
	9	100.2	60.0	59.4	17.1	36.8	4.39	1.53	2.50	2.98	0.77	4.91	39.8	1.48	43.0
	10	101.9	61.0	62.2	17.9	38.8	4.36	1.52	2.46	2.98	0.79	4.99	46.7	1.76	42.9
	11	103.4	61.8	64.6	18.6	40.8	4.31	1.51	2.42	2.97	0.81	5.04	53.7	2.03	42.5
	12	104.8	62.6	66.7	19.4	42.9	4.26	1.49	2.38	2.96	0.82	5.05	59.9	2.28	42.0
	13	105.9	63.4	68.5	20.0	44.9	4.21	1.48	2.34	2.96	0.82	5.05	64.9	2.46	41.3
	14	106.8	64.0	70.2	20.7	46.9	4.17	1.47	2.31	2.95	0.83	5.03	67.8	2.57	40.6
	15	107.5	64.6	71.7	21.3	49.0	4.16	1.47	2.30	2.94	0.82	5.00	68.1	2.57	40.0
	16	107.7	65.1	73.1	21.8	51.0	4.18	1.48	2.30	2.93	0.82	4.96	65.2	2.45	39.5
	17	107.7	65.5	74.5	22.3	53.0	4.25	1.51	2.31	2.93	0.81	4.93	58.3	2.18	39.2
	18	107.2	65.8	75.9	22.8	55.0	4.37	1.56	2.35	2.92	0.79	4.91	46.8	1.74	39.1
SD		8.67	7.78	10.17	4.15	18.31	0.75	0.32	0.66	0.75	0.37	0.40	30.71	1.24	6.62
N		9199	9113	11 039	10 907	4865	9526	9331	6297	9286	6136	5440	5549	5347	5612
						Boys									
Mean	6	95.8	58.0	51.2	15.1	24.6	4.39	1.48	2.51	2.83	0.65	4.51	21.9	0.78	46.1
	7	97.6	58.7	54.3	15.7	27.4	4.34	1.56	2.50	2.82	0.67	4.71	25.2	0.92	46.5
	8	99.4	59.4	57.2	16.4	29.7	4.30	1.60	2.47	2.83	0.69	4.86	29.8	1.11	47.0
	9	101.2	60.0	59.9	17.0	31.7	4.26	1.60	2.43	2.84	0.71	4.98	35.2	1.33	47.4
	10	103.0	60.7	62.6	17.7	33.3	4.22	1.59	2.38	2.85	0.73	5.06	41.0	1.57	47.9
	11	104.9	61.3	65.1	18.3	34.5	4.18	1.55	2.32	2.88	0.74	5.12	46.8	1.80	48.3
	12	106.7	62.0	67.4	19.0	35.3	4.13	1.50	2.27	2.91	0.76	5.15	52.2	2.02	48.8
	13	108.5	62.6	69.7	19.6	35.7	4.09	1.44	2.23	2.94	0.78	5.17	56.7	2.21	49.2
	14	110.3	63.3	71.8	20.3	35.7	4.05	1.39	2.19	2.99	0.80	5.17	60.1	2.35	49.6
	15	112.1	63.9	73.8	20.9	35.3	4.01	1.35	2.17	3.03	0.81	5.16	61.7	2.43	50.1
	16	113.9	64.6	75.6	21.6	34.6	3.96	1.32	2.17	3.09	0.83	5.15	61.4	2.42	50.5
	17	115.7	65.2	77.4	22.2	33.4	3.92	1.32	2.20	3.15	0.85	5.14	58.6	2.31	51.0
	18	117.5	65.9	78.9	22.9	31.8	3.88	1.34	2.25	3.22	0.87	5.13	52.9	2.10	51.4
SD		9.32	8.06	10.60	3.99	16.71	0.74	0.32	0.64	0.80	0.40	0.41	32.09	1.36	7.74
N		9046	8909	11 090	10 903	4848	9510	9307	6355	9262	6194	5539	5645	5450	5702

BMI: body mass index; CRF: cardiorespiratory fitness; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment; LDL-C: low-density lipoprotein; N: number of observations; SBP: systolic blood pressure; SD: standard deviation; Sum4Skin: sum of four skinfolds; TC: total cholesterol; TC:HDL-C: total cholesterol: high-density lipoprotein cholesterol ratio; TG: triglyceride; WC: waist circumference.

showed the lowest correlation with other risk scores, mean $r = 0.65$, whereas the overall correlation between all cluster scores was considerably higher, mean $r = 0.85$.

4. Discussion

The present study provides reference values for cardiometabolic risk factors in a large sample of European and American 6- to 18-year-old children. Future studies investigating prevalence of cardiometabolic risk in children or associations with, for example, lifestyle factors, may use the present data as a common reference when calculating and interpreting their results. First, the equations given in Table 3 can be used to calculate age-predicted reference values for individuals. Second, standardized scores can then be calculated for each individual variable by using the following equation: z-score = $[(x-\bar{x})/SD\bar{x}]$, where the age-predicted reference value is used as the mean ($\bar{x}$). Third, a mean-clustered risk score can be derived using these z-scores. A practical example of how to calculate comparable cluster risk scores is provided in the Supplementary material (Table D). Substituting sample-specific mean values with the age-predicted reference values and SDs, as suggested herein, will substantially increase the utility of the z-score approach in pediatric epidemiological research. This approach makes it possible to compare individual- and study-level data for single and clustered risk scores with representative groups of European and American children and can be used in both clinical and research settings. Further, the strong correlations found between different combinations of variables constituting clustered cardiometabolic risk scores suggests that it is possible to compare cardiometabolic cluster risk scores of different cohorts regardless of the ensemble of components included.

To our knowledge, only a few studies present the possibility to standardize pediatric risk variables for use in epidemiological work and analysis [40,41]. The fourth report from the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents provides reference values for systolic blood pressure derived from data from more than 60 000 American children [40]. Gurka and colleagues [41] have suggested a standardized equation to calculate continuous risk scores based on 4174 American children (NHANES) aged 12–19 years, comprising five cardiometabolic risk variables. The approach provides gender- and racial/ethnic-specific risk scores, but does not consider the effect of age on cardiometabolic risk [42]. Further, the scores are derived from factor analyses, which depend on measurement error and short time fluctuations within each risk factor, rather than the contribution of risk itself. Finally, the ability of Gurka's clustered risk score to predict type 2 diabetes and carotid intima media thickness has been investigated; however, the score did not show increased predictive utility compared to other continuous cluster scores [38].

Our study extends previous works by including a large international sample, and providing transparent methods to standardize up to 14 different cardiometabolic risk factors for monitoring, profiling, and surveillance of CVD risk in children during growth and maturation. The generalizability of the reference values is, nonetheless, limited by the sampling procedures and methodology within each original study. In general, all comparison between studies of cardiometabolic risk factors

Table 2
Reference values for single risk factors by age and gender (*log-transformed values*).

	Age (years)	WC	BMI	Sum4Skin	TC:HDL-C ratio	TG	Insulin	HOMA score
				Girls				
Mean	6	3.91	2.70	3.36	1.07	−0.45	3.04	−0.42
	7	3.97	2.74	3.41	1.07	−0.42	3.18	−0.21
	8	4.03	2.79	3.46	1.06	−0.38	3.34	−0.01
	9	4.08	2.83	3.51	1.06	−0.36	3.50	0.19
	10	4.12	2.87	3.56	1.06	−0.33	3.66	0.37
	11	4.16	2.91	3.61	1.06	−0.31	3.81	0.53
	12	4.19	2.95	3.66	1.05	−0.30	3.94	0.67
	13	4.22	2.98	3.71	1.05	−0.29	4.04	0.77
	14	4.24	3.01	3.76	1.05	−0.29	4.10	0.82
	15	4.26	3.04	3.81	1.05	−0.29	4.11	0.83
	16	4.28	3.06	3.86	1.04	−0.29	4.07	0.78
	17	4.29	3.08	3.91	1.04	−0.30	3.96	0.66
	18	4.31	3.09	3.96	1.04	−0.32	3.77	0.48
SD		0.13	0.18	0.38	0.24	0.42	0.50	0.53
				Boys				
Mean	6	3.95	2.74	3.16	1.01	−0.51	2.93	−0.50
	7	4.00	2.77	3.24	1.01	−0.49	3.06	−0.32
	8	4.05	2.80	3.30	1.01	−0.47	3.20	−0.13
	9	4.09	2.83	3.36	1.01	−0.45	3.35	0.05
	10	4.13	2.86	3.40	1.02	−0.43	3.50	0.23
	11	4.17	2.89	3.44	1.02	−0.40	3.65	0.39
	12	4.20	2.93	3.46	1.03	−0.38	3.78	0.52
	13	4.23	2.96	3.47	1.04	−0.36	3.88	0.63
	14	4.26	2.99	3.48	1.06	−0.34	3.96	0.71
	15	4.29	3.02	3.47	1.07	−0.32	3.99	0.74
	16	4.31	3.05	3.45	1.09	−0.29	3.98	0.73
	17	4.33	3.09	3.42	1.11	−0.27	3.92	0.67
	18	4.35	3.12	3.39	1.13	−0.25	3.79	0.54
SD		0.14	0.17	0.40	0.25	0.44	0.55	0.59

BMI: body mass index; HOMA: homeostatic model assessment; log: logarithmically; SD: standard deviation; Sum4Skin: sum of four skinfolds; TC:HDL-C: total cholesterol: high-density lipoprotein cholesterol ratio; TG: triglyceride; WC: waist circumference.

are limited by the use of different protocols and lack of standardized methods, which also pose a challenge when pooling data from different cohorts. International consensus of standard methods to obtain cardiometabolic risk factors are therefore a future challenge in this area of research. Further, we did not account for ethnic diversity, which might influence CVD risk factor levels [43]. However, the present study provides a common reference material to define pediatric cardiometabolic risk and on data including different races and ethnic subgroups from both Europe and USA (≈60% Caucasian (Supplementary Table C)). If risk factor levels differ among subgroups, these subgroups will have higher or lower standardized risk scores than others, but still be comparable to the reference material and other studies of the same subgroups using these reference values for standardization. Thus, we argue that the proposed reference material in any case will facilitate comparison across studies and over time, and contribute to move the research field of childhood cardiometabolic health forward.

We investigated linear, quadratic, and cubic trends with age to fit the best equations for each of the 14 cardiometabolic risk variables. Allowing data to fluctuate with age does, however, increase the possibility of overfitting data. Consistent with previous studies, some risk factors do not change linearly over time and differ between genders [20,21,40,44,45], most likely because of biological changes related to age and maturation. These changes can potentially influence the stability and accuracy of the MetS diagnosis [42]. For instance, puberty has a considerable impact on fat distribution, insulin sensitivity and secretion [45], and levels of TG have been shown to increase while levels of HDL-C decrease [20]. Although we lack a complete pathophysiological understanding of all cardiometabolic risk factors, and the timing and influence of puberty and growth are unclear, we consider the use of non-linear trends in growing children an appropriate approach.

While residuals for most variables did not vary with age, residuals increased with age for the skewed variables: WC, BMI, skinfold thickness, insulin, and HOMA scores. After log transformation, the variation of residuals was considerably less. When comparing standardized cluster risk scores (consisting of SBP and the log-transformed variables TC:HDL-C ratio, TG, HOMA score, and WC) using either constant or age-specific SD values within the youngest and oldest age groups, results were similar (ICC ≥ 0.99). We therefore choose to use the same robust SDs for all age groups to allow for a simpler application of algorithms.

Continuous cluster risk scores have been used frequently in studies of pediatric cardiometabolic risk [18,38] and have been shown to be associated with levels of PA and CRF [46], and to predict adult type 2 diabetes and carotid artery intima media thickness [38]. When comparing the predictive ability of continuous approaches against a dichotomized MetS definition, less evidence exists. Nevertheless, these studies show that the continuous risk score is either equal to [38] or superior to a dichotomized risk score in estimating children's future cardiovascular health [47]. Still, the predictive ability of later cardiometabolic risk and disease from clustered cardiometabolic risk scores in children has only shown to be moderate [38]. More longitudinal studies following children over time are needed to illuminate this area of research and to increase our understanding about the best approach to predict increased risk of cardiometabolic diseases from an early age.

The identification of “high-risk individuals” can vary greatly in the same group of children depending on the approach applied [17]. Andersen and colleagues identified less than 1% of children at risk using the IDF definition [19]. When using a clustered z-score approach in the same group of children, the risk group was extended to approximately 16% [19]. This latter group of children was characterized by having 3 or more clustered risk variables; in other words, having an unhealthy

Table 3
Regression equations by age for single cardiometabolic risk factors in girls and boys.
Equations for actual units are shown for all included variables, whereas equations for log-transformed values are shown only for skewed variables.

	Girls						Boys					
	γ-intercept	β-coefficient			SD	ICC	γ-intercept	β-coefficient			SD	ICC[a]
		age	*age²*	*age³*				*age*	*age²*	*age³*		
					Absolute values							
SBP	84.085419	1.352664	0.100390	−0.005781	8.6722095	0.09	84.930208	1.810944			9.3180619	0.08
DBP	48.251195	1.642089	−0.036912		7.7788888	0.24	54.131674	0.652442			8.0580317	0.26
WC	6.923239	9.461851	−0.494561	0.010098	10.1742481	0.14	30.320353	3.876927	−0.065298		10.6005333	0.06
BMI	7.843299	1.219271	−0.021625		4.1473040	0.06	11.196837	0.650082			3.9862129	0.05
Sum4Skin	18.512255	2.029718			18.3129132	0.10	−0.308554	5.330204	−0.196941		16.7136902	0.06
TC	2.327413	0.613717	−0.057694	0.001662	0.7496077	0.07	4.641488	−0.042366			0.7383655	0.05
HDL-C	0.730070	0.230285	−0.021182	0.000608	0.3196003	0.07	−0.212351	0.504949	−0.044087	0.001157	0.3210060	0.06
LDL-C	2.219779	0.148092	−0.018060	0.000569	0.6603341	0.08	2.006579	0.200585	−0.023807	0.000745	0.6446768	0.08
TC:HDL-C	3.044685	−0.006873			0.7540697	0.12	2.993067	−0.047512	0.003353		0.8018310	0.11
TG	0.407089	0.059863	−0.002143		0.3723611	0.09	0.547833	0.017777			0.4018758	0.07
Glucose	1.493865	0.742463	−0.050012	0.001072	0.3981106	0.16	2.171775	0.588765	−0.037923	0.000797	0.4133370	0.24
Insulin	84.837643	−25.575818	3.268078	−0.109149	30.7104507	0.09	50.370236	−14.543330	2.041363	−0.068083	32.0871352	0.06
HOMA	2.425747	−0.802842	0.112573	−0.003893	1.2376530	0.07	1.861164	−0.569878	0.081108	−0.002707	1.3600340	0.05
CRF	20.809510	5.889239	−0.489963	0.012177	6.6184862	0.19	43.455681	0.441795			7.7436990	0.13
					Natural log-transformed values							
WC	3.340392	0.126343	−0.005788	0.0000974	0.1323175	0.15	3.609076	0.065647	−0.001350		0.1359390	0.07
BMI	2.474502	0.028888	0.002023	−0.000096	0.1801600	0.06	2.544058	0.031888			0.1749271	0.05
Sum4Skin	3.054577	0.050292			0.3769964	0.13	2.502077	0.141056	−0.005106		0.3981823	0.08
TC:HDL-C	1.085626	−0.002610			0.2351238	0.14	1.075531	−0.016854	0.001108		0.2462190	0.11
TG	−0.782962	0.069081	−0.002402		0.4154836	0.11	−0.643920	0.021832			0.4436847	0.08
Insulin	3.021447	−0.178175	0.039234	−0.001501	0.5043443	0.09	2.925177	−0.161127	0.034727	−0.001283	0.5538751	0.10
HOMA	−1.257847	0.033936	0.024693	−0.001179	0.5344390	0.07	−1.210175	0.018537	0.022704	−0.001018	0.5862900	0.07

β: beta; BMI: body mass index; CRF: cardiorespiratory fitness; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment; ICC: intra-class coefficient; log: logarithmically; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; SD: standard deviation; Sum4Skin: sum of four skinfolds; TC: total cholesterol; TC:HDL-C: total cholesterol:high-density lipoprotein cholesterol ratio; TG: triglyceride; WC: waist circumference; γ = regression.

[a] ICC's represents the cluster effect among the cohorts. All models significant, $p \leq 0.001$.

metabolic profile and an increased risk of later CVD. Identifying individuals at risk as early as possible is critical for prevention purposes. Importantly, children that exhibit only *increased* risk could go undetected when applying dichotomized definitions of the MetS, and prevention strategies would be unable to offer guidance and support for lifestyle changes. Further, dichotomization also makes it impossible to measure small and moderate changes in the degree of risk. Yet, dichotomization has its advantages as a diagnostic tool in medical and clinical settings to define individuals at risk, but it is imperative to establish thresholds using relevant biological information. For example, Andersen et al. [19] suggested a composite z-score cutoff point to be 0.39, to identify children at increased cardiometabolic risk (15.7% with clustering of ≥3 risk factors). Using a more conservative approach, a composite z-score cutoff point of 0.85 (≥4 risk factors) identified 6.2% children to be at risk. These thresholds could be considered when aiming at determine children at increased cardiometabolic risk. Nevertheless, these and other suggested cut-off values must be validated in long-term follow-up studies of large samples of children followed into adulthood when metabolic diseases emerge.

The optimal ensemble of risk variables in the MetS is not fully known, albeit most researchers in the field agree that hypertension, adiposity, dyslipidemia (cholesterol and triglycerides), and insulin resistance are the physiological risk components to be considered in a clustered risk score. The bivariate correlation analyses (Table 4) suggest that the cluster scores representing these four risk components are strongly related. Thus, future studies that standardize their risk variables according to the present reference material have the flexibility to calculate a clustered mean risk score of their own choice, or available components, and still be able to compare their results. Even simple non-invasive risk scores consisting of SBP, adiposity, and CRF have shown high sensitivity and specificity when compared with other definitions including all risk components [48]. However, one of the simplest cluster-risk scores included in this study, consisting of DBP, TC, and HDL-C, produced the poorest correlation of all cluster scores. It might be of minor importance which single risk factors are included, as long as at least one of the *sine qua non* variables in the MetS, insulin resistance or obesity [2,8], is represented. Cardiorespiratory fitness is not included in the description of the "original" risk components, but low CRF in adults has been shown to be one of the strongest determinants of overall mortality and risk of death caused by CVD and thus poses a major challenge for public health [49,50]. In children, the evidence is less clear, but accumulating research shows an inverse relationship between levels of CRF and clustering of cardiometabolic risk factors, as well as risk of later CVDs [46]. Therefore, reference values for CRF are also presented and may be considered as an additional and important risk factor in any cardiometabolic cluster risk score.

We recommend that future studies apply the reference values, as suggested herein, to increase standardization of calculations and definitions used to identify cardiometabolic risk in children. This unified approach to define and understand cardiometabolic risk in the pediatric population will substantially reduce confusion and allow for increased knowledge in this important field of research.

In summary, this study presents the so far largest international reference material of cardiometabolic disease risk factors for the pediatric population. We suggest using this material as a common standard to define age- and gender-specific cardiometabolic risk in children. This approach makes continuous single risks factors and clustered cardiometabolic disease risk scores comparable to the reference material itself and comparable to cardiometabolic disease risk values in future studies adapting the same strategy, which is a significant step forward in the field of pediatric epidemiology.

Table 4
Matrix of bivariate correlations of continuous cardiometabolic cluster score approaches[a] used in pediatric populations.

		Mean (SD)	Correlations[b]															
Cluster scores	**Girls**		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	DBP + TC + HDL-C	0.04 (0.51)	1															
2	Average (SBP + DBP) + HDL-C + TG + Glucose + WC	−0.03 (0.50)	0.63	1														
3	Average (SBP + DBP) + HDL-C + TG + HOMA + S4S	−0.01 (0.56)	0.60	0.88	1													
4	Average (SBP + DBP) + HDL-C + TG + Glucose + Insulin + WC	−0.03 (0.50)	0.57	0.96	0.93	1												
5	MAP + HDL-C + TG + WC	−0.05 (0.53)	0.70	0.91	0.89	0.86	1											
6	MAP + HDL-C + TG + HOMA + BMI	−0.03 (0.53)	0.62	0.89	0.96	0.95	0.91	1										
7	MAP + HDL-C + TG + Glucose + WC	−0.03 (0.50)	0.64	1.00	0.88	0.95	0.91	0.89	1									
8	MAP + HDL-C + TG + HOMA + WC + TC	−0.02 (0.47)	0.76	0.85	0.90	0.90	0.87	0.92	0.85	1								
9	MAP + HDL-C + TC:HDL-C + TG + Glucose + S4S	−0.01 (0.54)	0.74	0.93	0.91	0.89	0.86	0.87	0.93	0.86	1							
10	SBP + TC:HDL-C + Insulin	−0.01 (0.64)	0.66	0.69	0.80	0.79	0.68	0.83	0.66	0.86	0.71	1						
11	SBP + HDL-C + TG + Insulin + BMI	−0.05 (0.55)	0.53	0.83	0.94	0.90	0.86	0.97	0.81	0.89	0.81	0.88	1					
12	SBP + TC:HDL-C + TG + HOMA + WC	−0.05 (0.56)	0.65	0.84	0.91	0.91	0.85	0.93	0.83	0.97	0.83	0.91	0.94	1				
13	SBP + TC:HDL-C + TG + HOMA + S4S	−0.02 (0.59)	0.64	0.82	0.95	0.88	0.81	0.91	0.81	0.94	0.88	0.88	0.92	0.96	1			
14	SBP + TC:HDL-C + TG + HOMA + WC + CRF	−0.08 (0.54)	0.58	0.80	0.89	0.87	0.81	0.90	0.79	0.93	0.80	0.86	0.92	0.96	0.94	1		
15	SBP + TC:HDL-C + TG + HOMA + S4S + CRF	−0.05 (0.57)	0.57	0.78	0.92	0.84	0.77	0.88	0.76	0.89	0.84	0.83	0.89	0.92	0.96	0.97	1	
16	SBP + DBP + TG + HDL-C + Glucose + Insulin + BMI + WC + S4S	−0.01 (0.50)	0.57	0.91	0.93	0.93	0.86	0.91	0.91	0.86	0.86	0.74	0.87	0.87	0.89	0.87	0.87	1
Cluster scores	**Boys**																	
1	DBP + TC + HDL-C	0.03 (0.51)	1															
2	Average (SBP + DBP) + HDL-C + TG + Glucose + WC	−0.03 (0.50)	0.62	1														
3	Average (SBP + DBP) + HDL-C + TG + HOMA + S4S	−0.03 (0.55)	0.60	0.86	1													
4	Average (SBP + DBP) + HDL-C + TG + Glucose + Insulin + WC	−0.02 (0.50)	0.56	0.96	0.92	1												
5	MAP + HDL-C + TG + WC	−0.04 (0.53)	0.71	0.90	0.89	0.85	1											
6	MAP + HDL-C + TG + HOMA + BMI	−0.04 (0.52)	0.62	0.89	0.97	0.94	0.92	1										
7	MAP + HDL-C + TG + Glucose + WC	−0.02 (0.50)	0.64	1.00	0.86	0.95	0.90	0.88	1									
8	MAP + HDL-C + TG + HOMA + WC + TC	−0.04 (0.47)	0.75	0.84	0.91	0.90	0.87	0.93	0.84	1								
9	MAP + HDL-C + TC:HDL-C + TG + Glucose + S4S	−0.03 (0.53)	0.72	0.94	0.90	0.90	0.88	0.88	0.94	0.87	1							
10	SBP + TC:HDL-C + Insulin	−0.02 (0.64)	0.63	0.69	0.81	0.79	0.70	0.83	0.66	0.87	0.71	1						
11	SBP + HDL-C + TG + Insulin + BMI	−0.06 (0.56)	0.52	0.83	0.94	0.90	0.87	0.97	0.81	0.89	0.82	0.89	1					
12	SBP + TC:HDL-C + TG + HOMA + WC	−0.06 (0.56)	0.62	0.83	0.91	0.90	0.85	0.93	0.81	0.97	0.83	0.93	0.95	1				
13	SBP + TC:HDL-C + TG + HOMA + S4S	−0.06 (0.59)	0.61	0.80	0.95	0.87	0.82	0.91	0.79	0.94	0.86	0.90	0.93	0.97	1			
14	SBP + TC:HDL-C + TG + HOMA + WC + CRF	−0.10 (0.54)	0.56	0.78	0.89	0.86	0.81	0.90	0.76	0.92	0.80	0.87	0.92	0.96	0.95	1		
15	SBP + TC:HDL-C + TG + HOMA + S4S + CRF	−0.09 (0.57)	0.54	0.75	0.92	0.83	0.77	0.88	0.73	0.89	0.82	0.84	0.89	0.92	0.96	0.98	1	
16	SBP + DBP + TG + HDL-C + Glucose + Insulin + BMI + WC + S4S	−0.01 (0.50)	0.58	0.91	0.93	0.93	0.85	0.92	0.90	0.88	0.87	0.76	0.88	0.88	0.89	0.87	0.87	1

BMI: body mass index; CRF: cardiorespiratory fitness; HDL-C: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment; MAP: mean arterial pressure [DBP + (SBP-DBP/3)]; SD: standard deviation; S4S: sum of four skinfolds; TC: total cholesterol; TC:HDL-C: total cholesterol: high-density lipoprotein cholesterol ratio; TG: triglyceride; WC: waist circumference. *Log-transformed values:* WC, BMI, S4S, TG, TC:HDL-C, Insulin, and HOMA score.

[a] Clustered risk scores were constructed from examples of previously applied continuous cardiometabolic cluster scores in the pediatric population [18,38,39].

[b] All correlations significant at the $p \leq 0.001$ level (2-tailed). In total, 6471 (girls n = 3230, boys n = 3241) had valid data in all risk variables.

Conflicts of interest

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

Author contributions

LBA created the custom dataset, later updated by MS, comprising pooled data from multiple cohorts from Europe and United States. MS, LBA, GKR, and EAa developed the idea and design of the work. MS and EAa completed analyses, interpreted these data and drafted the manuscript. LBA and UE was helpful with statistical guidance when needed. LBA, LBS, JS-J, SAA, SK, and SLD planned/executed the original studies and acquired the databases. All authors have contributed to critical revisions and editing the manuscript, and have approved the final manuscript. MS and EAa are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.10.003.

References

[1] E. Kylin, Studien über das Hypertonie-Hyperglykämie-Hyperurikämie Syndrom [German], Zentralblatt für Innere Medizin (1923) 105–112.
[2] G.M. Reaven, Banting lecture 1988. Role of insulin resistance in human disease, Diabetes 37 (1988) 1595–1607.
[3] R. Kahn, J. Buse, E. Ferrannini, et al., The metabolic syndrome: time for a critical appraisal: joint statement from the American diabetes association and the European association for the study of diabetes, Diabetes Care 28 (2005) 2289–2304.
[4] K.G. Alberti, R.H. Eckel, S.M. Grundy, et al., Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; World heart federation; international atherosclerosis society; and international association for the study of obesity, Circulation 120 (2009) 1640–1645.
[5] K.G. Alberti, P.Z. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation, Diabet. Med.: J. Brit. Diab. Assoc. 15 (1998) 539–553.
[6] S.M. Grundy, H.B. Brewer Jr., J.I. Cleeman, et al., Definition of metabolic syndrome: report of the national heart, lung, and blood institute/American heart association conference on scientific issues related to definition, Circulation 109 (2004) 433–438.
[7] K.G. Alberti, P. Zimmet, J. Shaw, et al., The metabolic syndrome–a new worldwide definition, Lancet 366 (2005) 1059–1062.
[8] P. Zimmet, K.G. Alberti, F. Kaufman, et al., The metabolic syndrome in children and adolescents - an IDF consensus report, Pediatr. Diabetes 8 (2007) 299–306.
[9] R. Weiss, J. Dziura, T.S. Burgert, et al., Obesity and the metabolic syndrome in children and adolescents, N. Engl. J. Med. 350 (2004) 2362–2374.
[10] S. Cook, M. Weitzman, P. Auinger, et al., Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994, Arch. Pediatr. Adolesc. Med. 157 (2003) 821–827.
[11] C.J. Jolliffe, I. Janssen, Development of age-specific adolescent metabolic syndrome criteria that are linked to the Adult Treatment Panel III and International Diabetes Federation criteria, J. Am. Coll. Cardiol. 49 (2007) 891–898.
[12] E.S. Ford, Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence, Diabetes Care 28 (2005) 1769–1778.
[13] A.S. Gami, B.J. Witt, D.E. Howard, et al., Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies, J. Am. Coll. Cardiol. 49 (2007) 403–414.
[14] J. Cohen, The cost of dichotomization, Appl. Psychol. Meas. 7 (1983) 249–253.
[15] R.C. MacCallum, S. Zhang, K.J. Preacher, et al., On the practice of dichotomization of quantitative variables, Psychol. Methods 7 (2002) 19–40.
[16] J.W. Beckstead, T.M. Beckie, How much information can metabolic syndrome provide? An application of information theory, Med. Decis. Making 31 (2011) 79–92.
[17] A. Friend, L. Craig, S. Turner, The prevalence of metabolic syndrome in children: a systematic review of the literature, Metab. Syndrome Relat. Disord. 11 (2013) 71–80.
[18] J.C. Eisenmann, On the use of a continuous metabolic syndrome score in pediatric research, Cardiovasc. Diabetol. 7 (2008) 17.
[19] L.B. Andersen, J.B. Lauersen, J.C. Brond, et al., A new approach to define and diagnose cardiometabolic disorder in children, J. Diabet. Res. 2015 (2015) 539835.
[20] J.A. Morrison, P.M. Laskarzewski, J.L. Rauh, et al., Lipids, lipoproteins, and sexual maturation during adolescence: the Princeton maturation study, Metabolism 28 (1979) 641–649.
[21] A. Moran, D.R. Jacobs Jr., J. Steinberger, et al., Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females, Circulation 117 (2008) 2361–2368.
[22] J. Steene-Johannessen, E. Kolle, S.A. Anderssen, et al., Cardiovascular disease risk factors in a population-based sample of Norwegian children and adolescents, Scand. J. Clin. Lab. Invest. 69 (2009) 380–386.
[23] C. Riddoch, D. Edwards, A. Page, et al., The European youth heart study—-cardiovascular disease risk factorsin children: rationale, aims, study design, and validation of methods, J. Phys. Activ. Health (2005) 115–129.
[24] P.L. Kristensen, N.C. Moeller, L. Korsholm, et al., The association between aerobic fitness and physical activity in children and adolescents: the European youth heart study, Eur. J. Appl. Physiol. 110 (2010) 267–275.
[25] S. Kriemler, L. Zahner, C. Schindler, et al., Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial, BMJ 340 (2010) c785.
[26] U. Meyer, C. Schindler, L. Zahner, et al., Long-term effect of a school-based physical activity program (KISS) on fitness and adiposity in children: a cluster-randomized controlled trial, PloS One 9 (2014).
[27] A. Bugge, B. El-Naaman, M. Dencker, et al., Effects of a three-year intervention: the copenhagen school Child intervention study, Med. Sci. Sports Exerc. 44 (2012) 1310–1317.
[28] G. Zipf, M. Chiappa, K.S. Porter, et al., National health and nutrition examination survey: plan and operations, 1999-2010, Vital Health Stat 1 (2013) 1–37.
[29] L. Zahner, J.J. Puder, R. Roth, et al., A school-based physical activity program to improve health and fitness in children aged 6-13 years ("Kinder-Sportstudie KISS"): study design of a randomized controlled trial [ISRCTN15360785], BMC Publ. Health (2006) 6.
[30] CDC, Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. Questionnaires, Datasets, and Related Documentation; https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.
[31] M.J. Steiner, A.C. Skinner, E.M. Perrin, Fasting might not be necessary before lipid screening: a nationally representative cross-sectional study, Pediatrics 128 (2011) 463–470.
[32] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[33] D.R. Matthews, J.P. Hosker, A.S. Rudenski, et al., Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.
[34] S. Eiberg, H. Hasselstrom, V. Gronfeldt, et al., Physical fitness as a predictor of cardiovascular disease risk factors in 6-to 7-year-old Danish children: the Copenhagen school-child intervention study, Pediatr. Exerc. Sci. 17 (2005) 161–170.
[35] R.R. Pate, C.Y. Wang, M. Dowda, et al., Cardiorespiratory fitness levels among US youth 12 to 19 years of age: findings from the 1999-2002 national health and nutrition examination survey, Arch. Pediatr. Adolesc. Med. 160 (2006) 1005–1012.
[36] L.A. Leger, D. Mercier, C. Gadoury, et al., The multistage 20 metre shuttle run test for aerobic fitness, J. Sports Sci. 6 (1988) 93–101.
[37] A. Mamen, G.K. Resaland, D.A. Mo, et al., Comparison of peak oxygen uptake in boys exercising on treadmill and cycle ergometers, Gazz. Med. Ital. 167 (2008) 15–21.
[38] C.G. Magnussen, S. Cheriyan, M.A. Sabin, et al., Continuous and dichotomous metabolic syndrome definitions in youth predict adult type 2 diabetes and carotid artery intima media thickness: the cardiovascular risk in young finns study, J. Pediatr. 171 (2016) 97–103 e103.
[39] G.K. Resaland, A. Mamen, C. Boreham, et al., Cardiovascular risk factor clustering and its association with fitness in nine-year-old rural Norwegian children, Scand. J. Med. Sci. Sports 20 (2010) e112–120.
[40] The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents, Pediatrics 114 (2004) 555–576.
[41] M.J. Gurka, C.L. Ice, S.S. Sun, et al., A confirmatory factor analysis of the metabolic syndrome in adolescents: an examination of sex and racial/ethnic differences, Cardiovasc. Diabetol. 11 (2012) 128.
[42] E. Goodman, S.R. Daniels, J.B. Meigs, et al., Instability in the diagnosis of metabolic syndrome in adolescents, Circulation 115 (2007) 2316–2322.
[43] G.A. Mensah, A.H. Mokdad, E.S. Ford, et al., State of disparities in cardiovascular health in the United States, Circulation 111 (2005) 1233–1241.
[44] S. Cook, P. Auinger, T.T. Huang, Growth curves for cardio-metabolic risk factors in children and adolescents, J. Pediatr. 155 (2009) S6 e15–26.
[45] A. Moran, D.R. Jacobs Jr., J. Steinberger, et al., Insulin resistance during puberty: results from clamp studies in 357 children, Diabetes 48 (1999) 2039–2044.
[46] J. Myers, P. McAuley, C.J. Lavie, et al., Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status, Prog. Cardiovasc. Dis. 57 (2015) 306–314.
[47] A.S. Kelly, J. Steinberger, D.R. Jacobs, et al., Predicting cardiovascular risk in young adulthood from the metabolic syndrome, its component risk factors, and a cluster score in childhood, Int. J. Pediatr. Obes. 6 (2011) e283–289.
[48] O. Lerum, E. Aadland, L.B. Andersen, et al., Validity of noninvasive composite scores to assess cardiovascular risk in 10-year-old children, Scand. J. Med. Sci. Sports 27 (2017) 865–872.
[49] P.T. Katzmarzyk, T.S. Church, S.N. Blair, Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men, Arch. Intern. Med. 164 (2004) 1092–1097.
[50] S.N. Blair, J.B. Kampert, H.W. Kohl 3rdet al., Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women, J. Am. Med. Assoc. 276 (1996) 205–210.

PAPER II

In review: PLOS ONE

Revision submitted: May 2019

Cardiometabolic risk factor levels in Norwegian children compared to international reference values: The ASK study

Mette Stavnsbo[1,2*], Turid Skrede[1,2], Eivind Aadland[1], Katrine N. Aadland[1], Mai Chinapaw[3], Sigmund A. Anderssen[1,2], Lars B. Andersen[1,2], Geir K. Resaland[4]

[1] Department of Sport, Food and Natural Sciences, Western Norway University of Applied Sciences, Sogndal, Norway.

[2] Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, Norway.

[3] Department of Public and Occupational Health, Amsterdam University Medical Centers, Vrije Universiteit Amsterdam, Amsterdam Public Health research institute, Amsterdam, The Netherlands.

[4] Center for physically active learning, Faculty of Education, Arts and Sports, Western Norway University of Applied Sciences, Sogndal, Norway.

*Corresponding author: Mette.Stavnsbo@hvl.no (MS)

Word count (plain text): 4301

Word count (abstract): 241

Abstract

Objective To investigate cardiometabolic risk factor levels in a group of Norwegian 10-year-old children compared to international values and examine the association between cardiorespiratory fitness (CRF) and the reference-standardized clustered risk score.

Methods 913 children (49% girls) were included from the Active Smarter Kids (ASK) study. Body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) to HDL-C ratio, triglyceride (TG), glucose, insulin, homeostatic model assessment (HOMA) score and CRF, were standardized according to international age-and sex-specific reference values.

Results The Norwegian children had significantly more favorable WC, DBP, glucose, HDL-C and CRF levels compared to the international reference values, but similar or less favorable levels of other cardiometabolic risk factors. CRF was the variable that differed the most from the international values (mean (95% CI) 1.20 (1.16 to 1.24) SD). The clustered risk score (excluding CRF) was higher in the Norwegian children, but decreased to below international levels when including CRF (mean (95% CI) - 0.08 (- 0.12 to - 0.05) SD). CRF had a significant inverse association with the clustered risk score (excluding CRF) (β - 0.37 SD, 95% CI - 0.43 to - 0.31).

Conclusions Norwegian children have substantially higher CRF levels than international standards, and including CRF in clustered risk scores reduces overall risk in Norwegian children below that of international levels. CRF is associated with improved cardiometabolic health in children.

Introduction

The clustering of cardiometabolic risk factors (hypertension, dyslipidemia, adiposity, and glucose intolerance) has its origin in childhood [1] and can track into adulthood [2].

These risk factors constitute the metabolic syndrome (MetS), but the use of different measures and ensembles of risk factors and thresholds [3-5] hampers comparison between studies. Further, dichotomization of risk factors to define cardiometabolic risk have several limitations, especially when carried out in children [6]. The dichotomization of biological traits ignores the continuous nature of risk and decreases the available information and thus power in statistical analysis. Moreover, since cardiovascular diseases are usually not manifested in children, the thresholds for classifying children as "*healthy*" or "*at risk*" [1, 7-9] are adapted from definitions in adults and introduce an arbitrary insincerity.

A large international reference material for cardiometabolic risk factor values in children and adolescents was recently published to deal with these issues [10]. The reference values can be used to standardize single cardiometabolic risk factors, allowing to compare otherwise population specific continuous clustered risk scores directly to the reference material itself and to other studies adapting the same approach. Thus, the study by Stavnsbo & colleagues facilitate international comparisons of prevalence and trends in pediatric cardiometabolic risk.

Cardiorespiratory fitness (CRF) is a strong predictor of cardiometabolic disease and all-cause mortality in adults [11, 12]. In children, the evidence is less clear but CRF has been inversely associated with clustered cardiometabolic risk factors in an accumulating number of studies [13-17].

The primary aim of this paper was to investigate cardiometabolic risk factor levels in a group of Norwegian 10-year-old school-children as compared to international reference values. A secondary aim was to examine the association between CRF and the reference-standardized clustered risk score.

Materials and methods

Design and population

The present study is a cross-sectional analysis of baseline data from the Active Smarter Kids (ASK) study, a seven month cluster-randomized controlled trial conducted in the school year of 2014-2015 in Western Norway [18]. In total, 1129 5th graders (94% of those invited) from 57 schools participated in the ASK study We included 913 children (49% girls vs. 51% boys) age 10.2 (± 0.3) that had valid data in all cardiometabolic risk factors of interest. The ASK study design, sampling procedures and methodology is described in details elsewhere [18], thus, only a brief description of the relevant procedures are provided herein.

Blood sampling

An intravenous blood sample was collected from the children's antecubital vein after an overnight fast. Serum were obtained following a standardized protocol. An ISO-certificated laboratory analyzed the serum samples for traditional risk factors related to cardiometabolic diseases; insulin, glucose, triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and total cholesterol (TC). A TC to HDL-C (TC:HDL-C) ratio was calculated to represent dyslipidemia. Insulin resistance were defined by the homeostatic model assessment (HOMA)-score = [insulin (pmol/L) * glucose (mmol/L)]/135 [19].

Resting blood pressure

Systolic (SBP) and diastolic (DBP) blood pressure were measured by the Omron HEM-907 automated BP monitor (Omron Healthcare, Inc, Vernon Hills, IL, US). The device is validated according to the AAMI validation protocol [20] and to the validation criteria of the international protocol for BP measuring devices [21]. The children were measured in a quiet room after resting for ten minutes in a sitting position. Four measurements were taken with one-minute pauses in-between and the mean of the last three measurements was used for analyses. If the difference between measurements was

>5 mmHg, we obtained one extra measurement, in which case the mean of the last four was calculated and used for analyses.

Anthropometry and sexual maturity

BMI

Body mass was measured to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany) with children wearing light clothing or underwear (preferred) depending on the acceptance of the child. A portable Seca 217 (SECA GmbH, Hamburg, Germany) was used to measure stature to the nearest 0.1 cm with the barefooted child facing forward. Body mass index (BMI) ($kg*m^{-2}$) was calculated as weight (kg) divided by the height squared (m^2).

Waist circumference

Waist circumference (WC) was measured using an ergonomic measuring tape (Seca 201, SECA GmbH, Hamburg, Germany). Two measures were taken between the lowest rib and the iliac crest to the nearest 0.5 cm with the child's abdomen relaxed at the end of a gentle expiration. If the difference between measurements was greater than one cm, we obtained a new measurement until two results were ≤ 1 cm apart. The mean of the two closest measurements was used for analyses.

Maturity

Pubertal stage was self-assessed by the child using the Tanner scale in color pictures as proposed by Carel and Leger [22]. The children were given the standardized series of images with explanatory text in a private room. The children were asked to put a checkmark in the box below the picture that best represented their stage (1 to 5) of development for each component. We used breast and genital development for girls and boys, respectively, as a measure of pubertal stage.

Demographic characteristics

We obtained self-reported educational level from the children's parents/legal guardians to assess socio-economic status (SES). Parental education was categorized into three levels using the highest

educational level obtained by the mother or father: i) upper or lower secondary school, ii) university < four years, and iii) university ≥ four years.

Cardiorespiratory fitness

Cardiorespiratory fitness levels were measured using the validated Andersen test [23, 24], an intermittent field-running test [23]. All children were tested indoor on a wooden or rubber hall floor. In groups of 10–20 children, participants ran a 20 meter distance between one end-line to another for 15 seconds and stood still for another 15 seconds. Children touched the floor with one hand behind the line each time before they turned around. The test lasted for 10 minutes, and the total distance (meters) covered was registered as the test result. The Andersen test performance was converted into VO_{2peak} by the following equation; girls VO_{2peak} = 32.5793 + (0.0309 × distance (m)) – (0.2351 × body mass (kg)), boys VO_{2peak} = 27.1689 + (0.0397 × distance (m)) – (0.1698 × body mass (kg)) [27].

Ethics

Our procedures and methods conform to ethical guidelines defined by the World Medical Association's Declaration of Helsinki and subsequent revisions [25]. The study protocol was approved by the Regional Committee for Medical Research Ethics (Reference Number: 2012/2304). Written informed consent was obtained from children’s parents or legal guardians prior to commencement to the study.

Statistics

Before analyses, all values exceeding five standard deviations from the mean were excluded from the data material. Skewed variables were logarithmically transformed by the natural logarithm (ln); BMI, WC, TG, TC:HDL-ratio, insulin and HOMA. A linear mixed model and a generalized estimating equation model including school as a random effect were used to examine differences between sexes for the continuous and categorical variables, respectively. The characteristics are presented as means and standard deviations (SD), median and interquartile range (IQR), or numbers and

percentage (%). To enable comparison of single and clustered cardiometabolic risk factor values between Norwegian children and the international reference values, we standardized the following risk factors according to the age- and sex-specific reference values suggested by Stavnsbo et al. [10]; BMI (ln), WC (ln), SBP, DBP, LDL-C, HDL-C, TC:HDL-ratio (ln), TG (ln), glucose, insulin (ln), HOMA score (ln), and CRF (VO_{2peak}). Each single risk variable was standardized by sex using the following equation; reference-standardized variable (z-score) = $(x+\bar{x})/SD(\bar{x})$, where age-predicted reference values were used as the mean ($\bar{x}$), calculated from regression equations for the single cardiometabolic risk factors [10]. A mean clustered reference-standardized risk score was calculated by summing up the z-scores WC, SBP, TC:HDL-ratio, TG, and HOMA score and divide by five. A second mean clustered risk score was calculated including CRF (VO_{2peak} inversed) by summing up the same reference-standardized risk factors as described above and CRF, and divide by six [26].

The association between the Andersen test and the reference-standardized clustered risk score (excl. CRF) was explored using a linear mixed model; CRF as the independent variable, the reference-standardized clustered risk score as the dependent variable, and school as a random effect (to account for the cluster effect). Age, sex, pubertal stage and SES were included as covariates, but only sex and pubertal stage changed the estimates and were therefore included in an adjusted model. In line with previous studies [14, 15], we investigating if sex moderated the association between CRF and clustered cardiometabolic risk factors, testing the statistical additive assumption in linear regression analysis. Thus, sex differences were investigated by including the interaction CRF*sex. To produce interpretable beta coefficients, both CRF and the clustered risk score were standardized before analysis.

All analyses were conducted using IBM SPSS version 23 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). A p-value ≤ 0.05 was considered statistically significant in all analyses.

Results

Table 1 presents descriptive statistics of the study population. The majority of the children were pre-pubertal at baseline (88% girls and 89% boys) and 63-64% of both girls and boys had at least one parent with a professional bachelor degree (<4 years of higher education). There were no sex differences in mean age, height, BMI, WC, SBP, DBP or LDL.

Table 1 Descriptive statistics of the study population by sex

	Girls (*n* = 446)	Boys (*n* = 467)	
	Mean (±SD)/ median [Q1-Q3]/ n (%)	Mean (±SD)/ median [Q1-Q3]/n (%)	*p*-value
Age (yr)	10.2 (0.3)	10.2 (0.3)	0.885
Puberty (tanner) n (%)			0.001
Stage 1	99 (22.2)	169 (36.2)	
Stage 2	292 (65.5)	248 (53.1)	
Stage 3-5	52 (11.7)	48 (10.3)	
Missing	3 (0.6)	2 (0.4)	
Parents` education level n (%)			0.888
≤ Upper secondary school	148 (33.2)	146 (31.3)	
<4 years of university	122 (27.4)	141 (30.2)	
≥4 years of university	159 (35.6)	159 (34.0)	
Missing	17 (3.8)	21 (4.5)	
Weight (kg)	37.1 (8.3)	37.0 (7.9)	0.941
Height (cm)	142.5 (6.8)	143.1 (6.7)	0.111
BMI (kg/m^2)	17.3 [15.9-19.6]	17.2 [15.8-19.4]	0.379
WC (cm)	59.6 [56.0-65.3]	60.8 [57.3-65.8]	0.061
SBP (mm Hg)	105.3 (8.5)	105.3 (8.2)	0.669
DBP (mm Hg)	58.1 (6.3)	57.4 (6.1)	0.095
LDL-C (mmol/L)	2.52 (0.62)	2.50 (0.67)	0.615
HDL-C (mmol/L)	1.55 (0.35)	1.63 (0.34)	0.001
TC:HDL-ratio	2.82 [2.48-3.37]	2.72 [3.00-3.12]	0.001
TG (mmol/L)	0.73 [0.58-0.96]	0.65 [0.52-0.83]	<0.001
Glucose (mmol/L)	4.94 (0.33)	5.02 (0.32)	<0.001
Insulin (pmol/L)	52.8 [39.0-75.4]	45.4 [32.7-60.8]	<0.001
HOMA score	1.93 [1.37-2.83]	1.67 [1.19-2.29]	<0.001
Andersen test (m)	870.1 (84.7)	922.9 (111.7)	<0.001
Estimated VO_{2peak} (ml/kg/min)	50.49 (3.1)	57.08 (4.7)	<0.001
Clustered risk score	0.11 (0.68)	-0.10 (0.59)	<0.001

BMI; body mass index, CRF; cardiorespiratory fitness, DBP; diastolic blood pressure, HDL-C; high-density lipoprotein cholesterol, HOMA; homeostatic model assessment, LDL-C; low-density lipoprotein cholesterol, n; number, SBP; systolic blood pressure, SD; standard deviation, TC; total cholesterol, TG; triglycerides, WC; waist circumference. A *p*-value ≤0.05 was considered statistically significant.

Girls had a significantly higher TC:HDL-ratio, TG, insulin, and HOMA score than boys, but lower CRF levels. Overall, girls had a less favorable risk score profile, represented by a higher clustered risk score (standardized by population specific means and SDs) than boys *(p*<0.001).

Table 2 Reference-standardized risk values according to Stavnsbo et al. [10]

	Girls	Boys
Reference-standardized risk factors[a]	Mean (±SD)	Mean (±SD)
BMI	0.03 (0.88)	0.03 (0.87)
WC	-0.14 (0.89)	-0.10 (0.81)
SBP	0.36 (0.99)	0.20 (0.88)
DBP	-0.39 (0.81)	-0.43 (0.75)
LDL-C	0.10 (0.94)	0.20 (1.05)
HDL-C	0.10 (1.08)	0.16 (1.06)
TC:HDL-ratio	0.03 (0.99)	-0.02 (0.89)
TG	0.14 (0.95)	0.04 (0.80)
Glucose	-0.16 (0.84)	-0.13 (0.77)
Insulin	0.58 (1.00)	0.49 (0.81)
HOMA score	0.50 (0.99)	0.43 (0.82)
CRF	1.20 (0.56)	1.23 (0.66)
Clustered risk score [b]	0.18 (0.67)	0.11 (0.55)
Clustered risk score incl. CRF [b]	- 0.05 (0.62)	-0.11 (0.52)

BMI; body mass index, CRF; cardiorespiratory fitness, DBP; diastolic blood pressure, HDL-C; high-density lipoprotein cholesterol, HOMA; homeostatic model assessment, LDL-C; low-density lipoprotein cholesterol, SBP; systolic blood pressure, SD; standard deviation, TC; total cholesterol, TG; triglycerides, WC; waist circumference.

[a] Reference-standardized values = $(x+\bar{x})/SD(\tilde{x})$, where age-predicted reference values were used as the mean ($\tilde{x}$) [10].

[b] The clustered risk scores was calculated from the following reference-standardized variables; WC, SBP, TC:HDL-ratio, TG, and HOMA score, excluding and including CRF (VO_{2peak} inversed).

Table 2 and Fig 1 shows the standardized difference in cardiometabolic risk factors and clustered risk scores between Norwegian children and international reference values from children of the same age and sex. Cardiorespiratory fitness differed the most of all standardized variables from the international reference values, showing significantly more favorable levels in the Norwegian children (mean (95% CI) 1.20 (1.16 to 1.24) SD). The Norwegian children also had significantly more favorable WC, DBP, HDL-C, and glucose levels in comparison to the international reference population. Less favorable levels were found for SBP, LDL-C, TG, insulin and HOMA score compared to the international standards. BMI and TC:HDL-ratio was not significantly different from the reference values. The clustered risk score (excluding CRF) was significantly higher in the Norwegian children

compared to international values (mean (95% CI) 0.15 (0.11 to 0.19) SD). On the contrary, when including CRF as an additional risk factor, the mean clustered risk score decreased to below international levels (mean (95% CI) - 0.08 (- 0.12 to - 0.05) SD).

Fig 1. Reference-standardized cardiometabolic risk factors. Mean (95% CI) of the reference-standardized single risk factors and mean clustered risk scores excluding and including cardiorespiratory fitness (CRF) (inversed). Reference-standardized z-scores= $(x+\bar{x})/SD(\bar{x})$, where age-predicted reference value was used as the mean ($\bar{x}$) (10). The cardiometabolic clustered risk scores consisted of the following reference-standardized risk factors; WC, SBP, TG, TC:HDL-ratio, and HOMA score, excluding and including CRF (inversed).

Cardiorespiratory fitness was significantly inversely associated with the reference-standardized clustered risk score (excluding CRF) (β - 0.37 SD, 95% CI - 0.43 to - 0.31), adjusted by sex and pubertal stage. There were no significant moderating effect of sex for the association between CRF and cardiometabolic clustered risk. Fig 2 illustrates the inverse association between quartiles of CRF (quartile 1 represents the least fit children and quartile 4 the most fit children) and the cardiometabolic reference-standardized clustered risk score in girls and boys (*p for trend* < 0.001).

Fig 2. Association between quartiles of cardiorespiratory fitness and the reference-standardized clustered risk score. Mean (95% CI) of the reference-standardized clustered cardiometabolic risk score (excluding CRF) across quartiles of CRF. A higher clustered risk score indicates a less favorable cardiometabolic profile. Children in quartile 1 of CRF are the least fit and children in quartile 4 the fittest. *P* for trend < 0.001.

Discussion

This is the first study to produce standardized cardiometabolic risk values according to the international reference material for cardiometabolic risk factors in children published by Stavnsbo et al. [10]. This method makes it possible to compare otherwise sample-specific clustered risk scores directly to international age- and sex-specific cardiometabolic risk values. The Norwegian children in the present study had a higher clustered risk score of traditional risk factors than the international reference population, due to higher levels of SBP, HOMA score, and TG. However, CRF was considerably higher in the Norwegian children and including CRF in the clustered risk score decreased the score to below international values.

Children from Nordic countries have earlier exhibited higher SBP values compared to children from other European countries [27, 28], which the present study confirms. Elevated SBP levels have been associated with organ damage in children [29] and track moderately into adulthood, increasing the risk of subclinical atherosclerosis [30]. Systolic BP was included in the clustered risk score, although DBP was lower in the Norwegian children compared to the international reference values. Systolic BP has been argued to be the recommended measure in children due to a greater accuracy and reproducibility than measures of DBP [31] and is often used as a single component to represent BP levels in pediatric clustered risk scores [10]. In addition, SBP has shown to be a better predictor of cardiometabolic risk compared to DBP in adults [32] and the treatment of isolated systolic hypertension has effectively reduced morbid cardiovascular events and lowered mortality [33]. Norwegian adults, especially men, have also shown to have higher SBP than most other European countries and the US [34]. Dietary differences between countries could possibly impact these variations in BP [35]. However, methodological differences could also be a plausible explanation for these findings. For instance, approximately 50% of the total population included in the reference material consist from the National Health and Nutrition Examination Survey (NHANES) from the USA, where BP was measured by the mercury sphygmomanometer. The auscultation method using a

mercury sphygmomanometer is the “gold standard” for BP measures [36]. In the present study we used an oscillometric BP device to measure BP. When validated against the mercury sphygmomanometer, oscillometric BP devices have shown to significantly overestimate SBP in children (2.53 mmHg; 95% CI [0.57 to 4.50]) [37]. The difference between SBP values in the Norwegian 10-year-olds compared to the international age-comparable children was 2.48 mmHg (95% CI [1.93-3.02]). The accuracy, however, of BP measures also depends on other factors such as the surrounding environment in which the measures are completed, the measurement procedures, and the test personnel [38].

The children in the present study had significantly higher HOMA scores than the international reference population. HOMA score has been shown to be a good surrogate measure for insulin resistance in youth, when validated against the euglycemic-hyperinsulinemic clamp method [39]. HOMA score has also shown to be a better predictor for clustered cardiometabolic risk factors, than fasting glucose and insulin levels alone [40]. Fasting glucose is typically normal in children, and studies of the glycemic control show that children manage to regulate their blood sugar well despite severe insulin resistance [41]. During the last decades, prevalence rates of insulin resistance and type 2 diabetes in children and adolescents have been on the rise globally [42]. Comparing HOMA levels in the ASK study to population-based samples of 9-10-year-old Norwegian children from 2005-2006 [43], shows that Norwegian children may be following international trends. When glucose and insulin was standardized separately according to the international reference values, the ASK population had slightly lower glucose values, but considerably higher insulin levels than the reference population. A potential methodological challenge when comparing insulin levels between populations is the use of different kits for analysis, since the binding characteristics of the plates may result slightly different. Storage time of blood samples before analyzes also influence on insulin levels. As an example of this, the multicultural European Youth Heart Study (EYHS), included in the reference material, had their Portuguese blood samples stored for several years before analysis. The Danish samples were analyzed shortly after collection in a WHO-certified laboratory. A subgroup of the Danish samples

were reanalyzed to investigate the influence of the long storage time, showing a strong correlation between insulin values between the first and second analysis (r = 0.97), but a substantial decrease (≈ 50%) in insulin levels. Thus, insulin levels from EYHS-Portugal were corrected by storage time to apply to all other obtained values on insulin in the EYHS study. It is, nevertheless, plausible that differences in storage time of blood samples in the studies included in the reference material and in the ASK study, could contribute to the higher insulin values and HOMA score observed in the present study.

Pediatric dyslipidemia is associated with initiation and progression of atherosclerotic lesions [44] and has shown persistence over time [45], increasing the risk of early atherosclerosis and premature cardiovascular diseases. Triglyceride and TC:HDL ratio was included in the clustered risk score in the present study to represent dyslipidemia, and both have been widely used in clustered risk scores in pediatric populations [10]. Compared to the international reference population, TG levels were significantly higher in the Norwegian children. In stratified analysis, girls exhibited higher TG levels than the Norwegian boys. One explanation of this finding could be that TG concentrations are positively associated with sexual maturation [46] and girls have shown to enter puberty at younger ages than observed previously [47]. For instance, the Copenhagen Puberty Study found that thelarche among Danish girls in 2006 started nearly one year earlier than 15 years previously, independently of changes in BMI [48]. The Norwegian children did not differ from the international reference population in TC:HDL-ratio, despite significantly higher HDL-C levels. High levels of HDL-C are positively correlated with CRF, which was higher in the present study, and HDL-C has shown to have an anti-atherosclerotic, and to some extent also cardioprotective, effect [49]. Lipid and lipoproteins levels are, nonetheless, influenced by several environmental and genetic factors, such as diet [50] and apolipoprotein variants [51], which we were not able to control for.

Time trends in cardiometabolic risk factor levels in children and the fact that the reference values are based on pooled data from both European and US data, may explain some of the observed

differences between the Norwegian and international risk factor values in general. The reference material comprised studies conducted between 1999 and 2008, while baseline data from the ASK study was collected in 2014. For instance, obesity levels in children and adolescents have shown a significant linear increase among children and adolescents during recent decades in both Europe and the US [52, 53]. The prevalence of obesity in Europe has, nevertheless, not increased to the same extent as in the US. According to WHO [52], the mean prevalence of obesity among adolescents (11-, 13- and 15-year-olds) from 27 European countries was 4% in 2014. In comparison, the prevalence of obesity among US children aged 12-15 years in 2014 was 20% [53]. Further, WHO reported that the lowest level of obesity in Europe in 2014 was found among Norwegian adolescents and Norway was one of the only European countries where an overall decrease in obesity was observed in the years between 2002 and 2014 (although only significantly in 13-year-old boys) [52]. This might explain why the children in the present study had significantly lower WCs than the international reference children. The Norwegian children's BMI did, nevertheless, not differ from the reference population. Waist circumference was prioritized in the clustered risk score since WC in children, for instance, has shown to be a stronger predictor of type 2 diabetes than BMI [54, 55]. In adults, WC has shown to be a strong predictor of both type 2 diabetes and CVD mortality, independent of other traditional cardiometabolic risk components, such as BP, insulin resistance and cholesterol levels [56, 57]. Waist circumference is also strongly inversely associated with CRF in children [16] and CRF levels in the present study were significantly higher than in the reference population.

Among the examined variables, CRF (VO_{2peak}) clearly differed the most from the international values, 1.20 and 1.23 SDs in girls and boys, respectively. Thus, when we included CRF (inversed) in the clustered risk score, the risk score decreased considerably. Strong evidence exist for the association between high levels of CRF in childhood and a healthier cardiometabolic risk profile later in life [58]. In adults, low CRF has shown to be a stronger predictor of CVDs and all-cause mortality than other established risk factors, such as hypertension, type 2 diabetes and high cholesterol levels [11, 12, 59-61]. Furthermore, both epidemiological studies and clinical evidence show that CRF in addition to

other traditional cardiometabolic risk factors enhances the precision of predicting CVD morbidity and mortality [60]. Although different fitness tests were used to produce reference values for CRF, these fitness tests rely on solid validations to reflect absolute VO_{2peak} [10]. Further, VO_{2peak} in the present study was estimated from validated algorithms, but could possibly overestimate true VO_{2peak} values [24]. However, high CRF levels are in line with previous studies showing that Norwegian children have relatively high VO_{2peak} [43, 62]. In support of this findings, international comparisons of children´s PA levels show that Norwegian children are more physically active and spend more time in higher intensities than children from most other countries [63].

It is contradictory that the Norwegian children have higher clustered cardiometabolic risk levels despite lower WC and higher CRF, compared to international values. One would expect lower (healthier) cardiometabolic risk factor levels in a fit population. However, similar finding have also been shown in a previous study from Western Norway [16]. The reason for the discrepancy in these risk factor profiles is difficult to identify, but could be due to cultural or environmental factors or any of the reasons discussed earlier, such as diet, genetics and methodologically differences. The ASK population is both lean and fit, and despite having higher levels in some cardiometabolic risk factors compared to international values, these levels are still considered to be within a healthy range. Positively, general levels of the traditional risk factors as well as CRF seem to have improved in children from Western Norway during the last decade [16].

The present study found an inverse association between CRF and clustered cardiometabolic risk (r = - 0.37) in accordance with earlier findings [13-17]. In comparison, Andersen et al. [13] found a stronger association (r = - 0.49) than observed in the present study, while others have found weaker associations (r = - 0.31 to - 0.09) [15, 17]. Aadland and colleagues [14] recently showed that CRF measured by the Andersen test is a more accurate marker of cardiometabolic health compared to directly measured VO_{2peak} and time to exhaustion (TTE), determined from a graded treadmill protocol in Norwegian 10-year-old children. The standardized regression coefficient between the Andersen

test and clustered cardiometabolic risk in the present study was lower than found by Aadland et al. [14]; *r* = - 0.45. Still, the Andersen test in the present study performed slightly better than both VO_{2peak} and TTE presented by Aadland et al. [14] as a marker of cardiometabolic health. Cardiorespiratory fitness is not regarded as one of the traditional cardiometabolic risk components and has been proclaimed as an overlooked and underutilized risk factor for cardiometabolic diseases along with PA [59, 60]. The undisputable positive association of CRF with cardiometabolic health, however, strongly argues for the inclusion of CRF in clustered cardiometabolic risk scores.

The main strength of the present study was the use of international reference values to standardize the cardiometabolic risk factors. This approach makes it possible to directly compare otherwise population specific cluster risk scores to the reference material of international cardiometabolic risk values. More specifically, if other studies adapt the same standardization strategy as the present study, they could compare single and clustered risk scores both to the international reference values and the present study population. Thus, the strength of using the reference values will increase with its usage in different studies and populations, and will make it possible to look at secular trends in the future. Further, the relatively large population sample is a strength of the present study. However, the homogeneity within the group (i.e. rural children from one Norwegian county, primarily Caucasian and in a limited age-range), limits generalization of the results. The use of the Andersen aerobic fitness test to estimate VO_{2peak} could be a limitation of our findings, since the Andersen test is an indirect measure of CRF. However, the test has shown both validity and reliability in the target age-group [24, 64]. As discussed earlier, the Andersen test also performed better as a marker of cardiometabolic health than both a direct VO_{2peak} test and TTE [14]. Furthermore, this test is more feasible in large population studies than direct measurement of VO_{2peak}. The Andersen test is a suitable aerobic fitness test in children because the intermittent running reflects children's natural running pattern and because it does not stigmatize children with a low CRF level. Dietary status was not registered in the ASK study and poses a limitation to the present study, since nutrition is a contributing factor to cardiometabolic risk [65].

Conclusions

This study is the first to standardize cardiometabolic risk factor levels in children according to international reference values. In comparison with international values, Norwegian children had significantly more favorable WC, DBP, glucose, HDL-C and CRF levels, but similar or less favorable levels of other cardiometabolic risk factors. The clustered cardiometabolic risk score (excl. CRF) was higher in the Norwegian children compared to the reference population. However, the Norwegian children`s CRF levels were more than 1 SD higher than the international mean. Adding CRF to the clustered cardiometabolic risk score lowered the score to below international values. CRF was associated with cardiometabolic risk, with low fit children scoring significantly higher on clustered cardiometabolic risk factors than children with higher CRF levels.

Acknowledgements

The authors are thankful to the children and their families who participated in the ASK study, and also the teachers and principals at the 57 schools involved. We would like to acknowledge all test personnel, such as nurses, bioengineers, and students for their great effort during the period of data collection.

References

1. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;350:2362-74
2. Magnussen CG, Smith KJ, Juonala M. When to prevent cardiovascular disease? As early as possible: lessons from prospective cohorts beginning in childhood. Curr Opin Cardiol 2013;28:561-8
3. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53
4. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. Lancet 2005;366:1059-62
5. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C, American Heart A, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8
6. Titmuss AT, Srinivasan S. Metabolic syndrome in children and adolescents: Old concepts in a young population. J Paediatr Child Health 2016;52:928-34
7. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med 2003;157:821-7
8. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet 2007;369:2059-61
9. Jolliffe CJ, Janssen I. Development of age-specific adolescent metabolic syndrome criteria that are linked to the Adult Treatment Panel III and International Diabetes Federation criteria. J Am Coll Cardiol 2007;49:891-8
10. Stavnsbo M, Resaland GK, Anderssen SA, Steene-Johannessen J, Domazet SL, Skrede T, et al. Reference values for cardiometabolic risk scores in children and adolescents – suggesting a common standard. Atherosclerosis 2018;278:299-306
11. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002;346:793-801
12. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory Fitness as a Quantitative Predictor of All-Cause Mortality and Cardiovascular Events in Healthy Men and Women A Meta-analysis. Jama-J Am Med Assoc 2009;301:2024-35
13. Andersen LB, Bugge A, Dencker M, Eiberg S, El-Naaman B. The association between physical activity, physical fitness and development of metabolic disorders. Int J Pediatr Obes 2011;6 Suppl 1:29-34
14. Aadland E, Kvalheim OM, Rajalahti T, Skrede T, Resaland GK. Aerobic fitness and metabolic health in children: A clinical validation of directly measured maximal oxygen consumption versus performance measures as markers of health. Prev Med Rep 2017;7:74-6
15. Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S, et al. Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. Diabetologia 2007;50:1832-40
16. Resaland GK, Mamen A, Boreham C, Anderssen SA, Andersen LB. Cardiovascular risk factor clustering and its association with fitness in nine-year-old rural Norwegian children. Scand J Med Sci Sports 2010;20:e112-20
17. Bailey DP, Boddy LM, Savory LA, Denton SJ, Kerr CJ. Associations between cardiorespiratory fitness, physical activity and clustered cardiometabolic risk in children and adolescents: the HAPPY study. Eur J Pediatr 2012;171:1317-23
18. Resaland GK, Moe VF, Aadland E, Steene-Johannessen J, Glosvik O, Andersen JR, et al. Active Smarter Kids (ASK): Rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on children's academic performance and risk factors for non-communicable diseases. BMC Public Health 2015;15:709

19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9
20. White WB, Anwar YA. Evaluation of the overall efficacy of the Omron office digital blood pressure HEM-907 monitor in adults. Blood Press Monit 2001;6:107-10
21. El Assaad MA, Topouchian JA, Darne BM, Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit 2002;7:237-41
22. Carel JC, Leger J. Precocious puberty. New Engl J Med 2008;358:2366-77
23. Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. J Sports Med Phys Fitness 2008;48:434-7
24. Aadland E, Terum T, Mamen A, Andersen LB, Resaland GK. The Andersen aerobic fitness test: reliability and validity in 10-year-old children. Plos One 2014;9:e110492
25. WMA. Ethical Principles for Medical Research Involving Human Subjects. World Medical Association General Assembly, Helsinki. 1964
26. Andersen LB, Harro M, Sardinha LB, Froberg K, Ekelund U, Brage S, et al. Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). Lancet 2006;368:299-304
27. Andersen LB, Henckel P, Saltin B. Risk-Factors for Cardiovascular-Disease in 16-19-Year-Old Teenagers. Journal of Internal Medicine 1989;225:157-63
28. Wynder EL, Williams CL, Laakso K, Levenstein M. Screening for risk factors for chronic disease in children from fifteen countries. Prev Med 1981;10:121-32
29. Kollias A, Dafni M, Poulidakis E, Ntineri A, Stergiou GS. Out-of-office blood pressure and target organ damage in children and adolescents: a systematic review and meta-analysis. J Hypertens 2014;32:2315-31; discussion 31
30. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation 2008;117:3171-80
31. Beevers G, Lip GY, O'Brien E. ABC of hypertension. Blood pressure measurement. Part I-sphygmomanometry: factors common to all techniques. BMJ 2001;322:981-5
32. Mourad JJ. The evolution of systolic blood pressure as a strong predictor of cardiovascular risk and the effectiveness of fixed-dose ARB/CCB combinations in lowering levels of this preferential target. Vasc Health Risk Manag 2008;4:1315-25
33. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. Lancet 2016;387:957-67
34. Collaboration NCDRF. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. Lancet 2017;389:37-55
35. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med 1997;336:1117-24
36. Jones DW, Appel LJ, Sheps SG, Roccella EJ, Lenfant C. Measuring blood pressure accurately: new and persistent challenges. JAMA 2003;289:1027-30
37. Duncombe SL, Voss C, Harris KC. Oscillometric and auscultatory blood pressure measurement methods in children: a systematic review and meta-analysis. J Hypertens 2017;35:213-24
38. Tolonen H, Koponen P, Naska A, Mannisto S, Broda G, Palosaari T, et al. Challenges in standardization of blood pressure measurement at the population level. BMC Med Res Methodol 2015;15:33
39. Gungor N, Saad R, Janosky J, Arslanian S. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr-Us 2004;144:47-55
40. Andersen LB, Boreham CA, Young IS, Davey Smith G, Gallagher AM, Murray L, et al. Insulin sensitivity and clustering of coronary heart disease risk factors in young adults. The Northern Ireland Young Hearts Study. Prev Med 2006;42:73-7

41. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of Type 1 and Type 2 Diabetes Among Children and Adolescents From 2001 to 2009. Jama-J Am Med Assoc 2014;311:1778-86
42. Farsani SF, van der Aa MP, van der Vorst MMJ, Knibbe CAJ, de Boer A. Global trends in the incidence and prevalence of type 2 diabetes in children and adolescents: a systematic review and evaluation of methodological approaches. Diabetologia 2013;56:1471-88
43. Steene-Johannessen J, Kolle E, Anderssen SA, Andersen LB. Cardiovascular disease risk factors in a population-based sample of Norwegian children and adolescents. Scand J Clin Lab Invest 2009;69:380-6
44. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650-6
45. Nicklas TA, von Duvillard SP, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: the Bogalusa Heart Study. Int J Sports Med 2002;23 Suppl 1:S39-43
46. Bertrais S, Balkau B, Charles MA, Vol S, Calvet C, Tichet J, et al. Puberty-associated differences in total cholesterol and triglyceride levels according to sex in French children aged 10-13 years. Ann Epidemiol 2000;10:316-23
47. Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. Horm Res Paediatr 2012;77:137-45
48. Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. Pediatrics 2009;123:e932-9
49. Assmann G, Gotto AM, Jr. HDL cholesterol and protective factors in atherosclerosis. Circulation 2004;109:III8-14
50. Kwiterovich PO, Vining EPG, Pyzik P, Skolasky R, Freeman JM. Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children. Jama-J Am Med Assoc 2003;290:912-20
51. Dron JS, Hegele RA. Genetics of Triglycerides and the Risk of Atherosclerosis. Curr Atheroscler Rep 2017;19:31
52. WHO. Adolescent obesity and related behaviours: trends and inequalities in the WHO European Region, 2002–2014. Observations from the Health Behaviour in School-aged Children (HBSC) WHO collaborative cross-national study. . http://www.euro.who.int/__data/assets/pdf_file/0019/339211/WHO_ObesityReport_2017_v3.pdf; 2017.
53. Skinner AC, Ravanbakht SN, Skelton JA, Perrin EM, Armstrong SC. Prevalence of Obesity and Severe Obesity in US Children, 1999-2016. Pediatrics 2018
54. Franks PW, Hanson RL, Knowler WC, Moffett C, Enos G, Infante AM, et al. Childhood predictors of young-onset type 2 diabetes. Diabetes 2007;56:2964-72
55. Garnett SP, Baur LA, Srinivasan S, Lee JW, Cowell CT. Body mass index and waist circumference in midchildhood and adverse cardiovascular disease risk clustering in adolescence. American Journal of Clinical Nutrition 2007;86:549-55
56. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875-80
57. Wang YF, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. American Journal of Clinical Nutrition 2005;81:555-63
58. Ruiz JR, Castro-Pinero J, Artero EG, Ortega FB, Sjostrom M, Suni J, et al. Predictive validity of health-related fitness in youth: a systematic review. Brit J Sport Med 2009;43:909-23
59. Myers J, McAuley P, Lavie CJ, Despres JP, Arena R, Kokkinos P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status. Prog Cardiovasc Dis 2015;57:306-14

60. Ross R, Blair SN, Arena R, Church TS, Despres JP, Franklin BA, et al. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. Circulation 2016;134:e653-e99
61. Blair SN. Physical inactivity: the biggest public health problem of the 21st century. Br J Sports Med 2009;43:1-2
62. Resaland GK, Mamen A, Anderssen SA, Andersen LB. Cardiorespiratory fitness and body mass index values in 9-year-old rural Norwegian children. Acta Paediatr 2009;98:687-92
63. Cooper AR, Goodman A, Page AS, Sherar LB, Esliger DW, van Sluijs EMF, et al. Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). Int J Behav Nutr Phy 2015;12
64. Aadland E, Andersen LB, Lerum O, Resaland GK. The Andersen aerobic fitness test: New peak oxygen consumption prediction equations in 10 and 16-year olds. Scand J Med Sci Sports 2017
65. Funtikova AN, Navarro E, Bawaked RA, Fito M, Schroder H. Impact of diet on cardiometabolic health in children and adolescents. Nutr J 2015;14:118

Supporting information

S1_Dataset. Supplementary data file including all material underlying the present study.

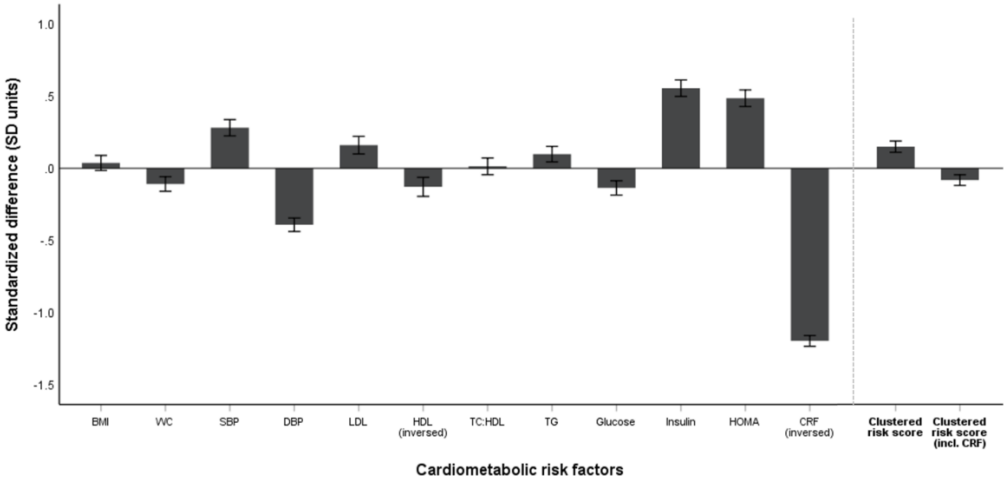

Fig 1. Reference-standardized cardiometabolic risk factors. Mean (95% CI) of the reference-standardized single risk factors and mean clustered risk scores excluding and including cardiorespiratory fitness (CRF) (inversed). Reference-standardized z-scores= x+x̄/sd (x̄), where age-predicted reference value was used as the mean (x̄) (10). The cardiometabolic clustered risk scores consisted of the following reference-standardized risk factors; SBP, ln (WC), ln (TG), ln (TC:HDL-c ratio), and ln (HOMA score), excluding and including CRF (inversed).

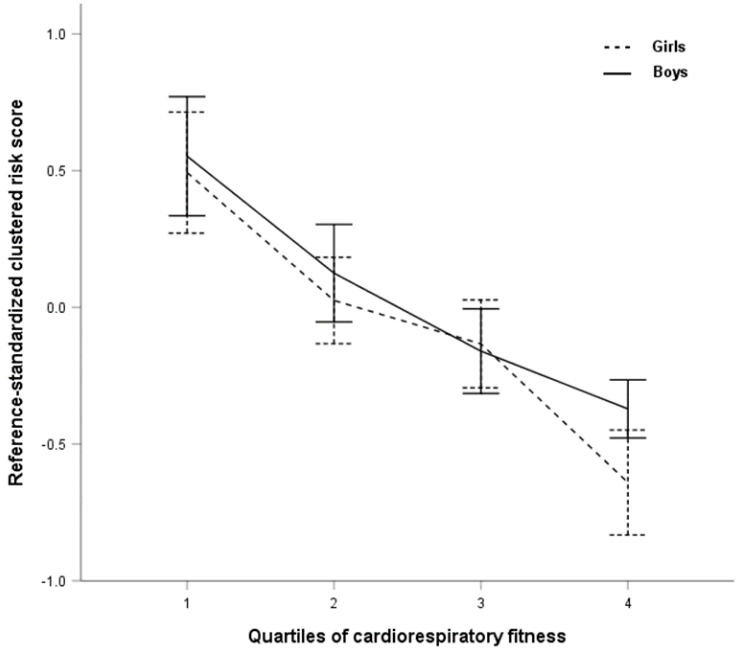

Fig 2. Association between quartiles of cardiorespiratory fitness and the reference-standardized clustered risk score. Mean (95% CI) of the reference-standardized clustered cardiometabolic risk score (excl. CRF) across quartiles of CRF. A higher clustered risk score indicates a less favorable cardiometabolic profile. Children in quartile 1 of CRF are the least fit and children in quartile 4 the fittest. P for trend < 0.001.

PAPER III

In review: Preventive Medicine

Submitted: April 2019

Effects of the Active Smarter Kids (ASK) physical activity intervention on cardiometabolic risk factors in children: a cluster-randomized controlled trial

Mette Stavnsbo[a,b*], Eivind Aadland[a], Sigmund A. Anderssen[a,b], Mai Chinapaw[c], Jostein Steene-Johannessen[b], Lars B. Andersen[a], Geir K. Resaland[d]

[a] Western Norway University of Applied Sciences. Department of Sport, Food and Natural Sciences. Sogndal, Norway

[b] Norwegian School of Sport Sciences, Department of Sports Medicine. Oslo, Norway

[c] Amsterdam University Medical Centers, Amsterdam Public Health research institute, Department of Public and Occupational Health. Amsterdam, the Netherlands.

[d] Western Norway University of Applied Sciences. Center for physically active learning, Faculty of Education, Arts and Sports. Sogndal, Norway.

*Corresponding author: Mette Stavnsbo, Sogn og Fjordane University College. PO Box 133, N-6856, Sogndal, Norway. Tel +47 57677647, email: Mette.Stavnsbo@hvl.no

Key words: Cluster RCT, CVD risk factors, cardiorespiratory fitness, physical activity, accelerometry, pediatric

Word count (plain text): 3456

Word count (abstract): 246

Abbreviations

ASK	Active Smarter Kids
BMI	body mass index
Cluster-RCT	cluster-randomized controlled trial
CRF	cardiorespiratory fitness
DBP	diastolic blood pressure
HDL	high-density lipoprotein
HOMA	homeostatic model assessment
LDL	low-density lipoprotein
MVPA	moderate-to-vigorous physical activity
PA	physical activity
PE	physical education
SBP	systolic blood pressure
SED	sedentary time
TC	total cholesterol
WC	waist circumference

Abstract

Objective To investigate the effect of a school-based cluster-randomized controlled trial of physical activity (PA) on single and clustered cardiometabolic risk factors.

Methods We included 1129 fifth grade children participating in the Active Smarter Kids (ASK) study conducted between November 2014 and June 2015, Sogn and Fjordane County, Norway. Cardiometabolic risk factors were waist circumference (WC), systolic blood pressure (SBP), total cholesterol (TC):high-density lipoprotein (HDL)-ratio, triglycerides (TG), homeostatic model assessment (HOMA)-score, and cardiorespiratory fitness (CRF). PA was measured by accelerometry. Between-group effects were analyzed using a linear mixed model accounting for the clustering of observations within schools.

Results No significant intervention effects were found for single or clustered cardiometabolic risk factors. However, in children with the most unfavorable baseline values, beneficial effects were found for SBP, TC:HDL ratio and the clustered cardiometabolic risk score. The effect of the intervention was also moderated by sex. Compared to boys, girls had a greater effect of the intervention on WC (p=0.03 for group*sex interaction) and CRF (p<0.001 for group*sex interaction).

Conclusions The majority of the children had initial high PA levels, thus limited potential for change, and we found no effects of the ASK school-based PA intervention on cardiometabolic risk in the total sample. However, subgroup analysis showed that girls had a more favorable effect on fatness and fitness than boys, and the data suggest that subgroups of children with the most unfavorable cardiometabolic risk profile and most in need of change can benefit from school-based PA interventions.

Trial registration number: Clinicaltrials.gov ID no.: NCT02132494

Introduction

The onset of cardiometabolic diseases are recognized to occur in early childhood (1-4). Physical activity (PA) contributes positively to cardiovascular health in addition to endocrine, cellular, skeletal and mental health (5). Importantly, children being regularly active in moderate-to-vigorous physical activity (MVPA) can improve their cardiorespiratory fitness (CRF) over time (6), which is a powerful marker of general health in children (7). Low CRF in adults is one of the strongest determinants of overall mortality and morbidity from cardiometabolic diseases (8,9). Unfortunately, children's PA levels decrease from early childhood to adolescence (10-12), and some studies indicate that patterns of both PA and inactivity track into adulthood (13,14). Thus, a major focus of public health initiatives has been to promote PA levels among children.

Children spent a substantial amount of time in school, making school an influential setting for children's development during their first two decades of life (15). Therefore, the World Health Organization, among others (16,17), has emphasized increased PA in schools as an ideal environment for public health interventions. School-based interventions of mandatory PA have many advantages; all children are included, which ensures PA for all in a non-stigmatizing way regardless of gender, age, weight status, fitness level, parent's socioeconomic status, and or attitude towards PA. Furthermore, the school system offers a stable schedule and can provide perpetuity of increased habitual activity from early in life. However, PA interventions in school children have shown conflicting results on cardiometabolic risk factors (18-21). Notably, the children most in need might have the greatest effects (22), which supports a population approach for reaching high-risk children.

The aim of this paper was to investigate the effect of a school-based PA cluster-RCT on single and clustered cardiometabolic risk factors in 10-year-old children. The intervention was conducted in a real-world setting and consisted of a curriculum-prescribed PA intervention delivered by classroom teachers.

Materials and Methods

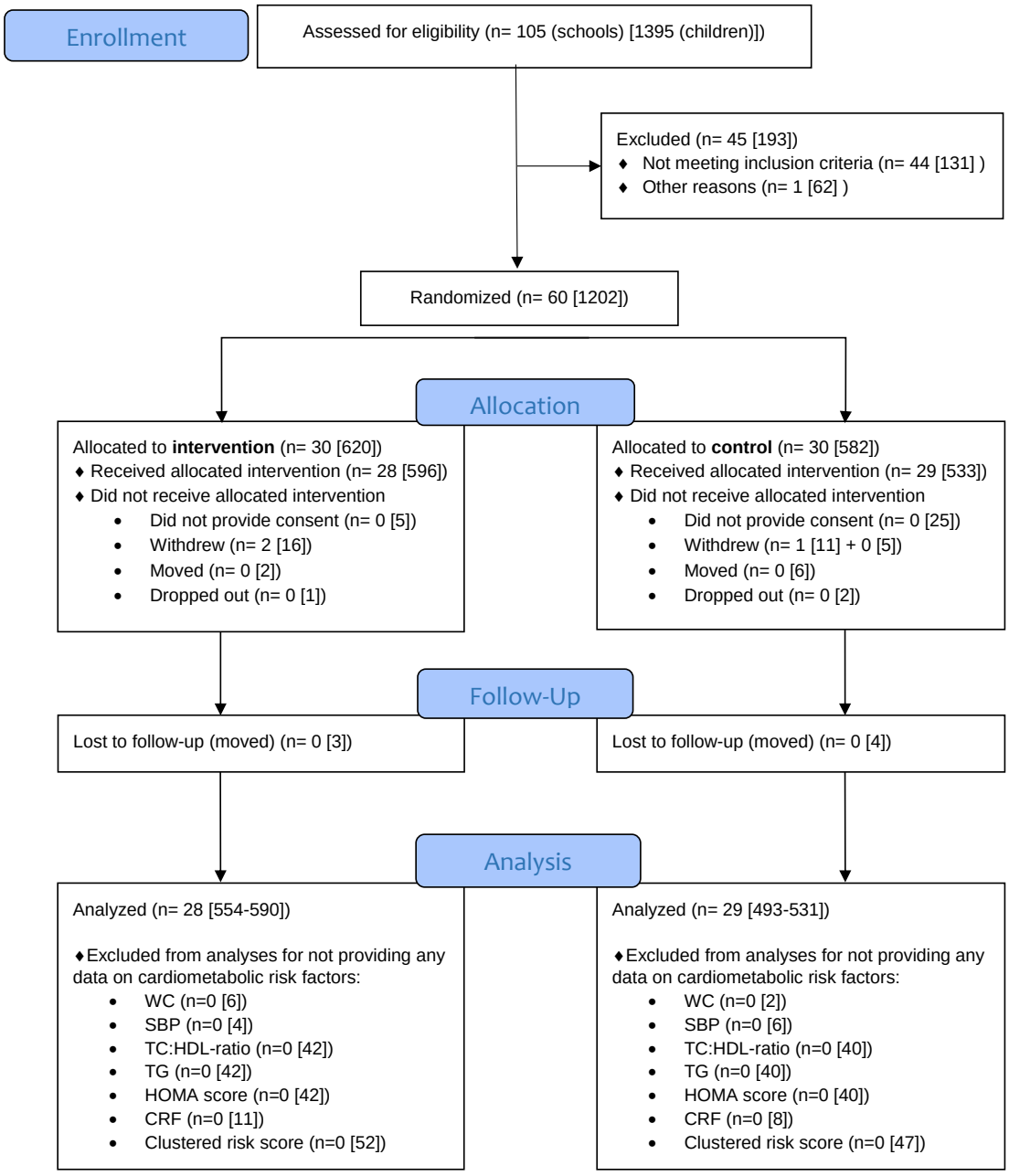

Fig. 1 Cohort flowchart of the enrollment, allocation, and follow-up of ASK children and final included population in the statistical analysis. The numbers in ( ) and [ ] refer to the number of schools and the number of children, respectively.

Abbreviations: CRF: cardiorespiratory fitness; HDL: high-density lipoprotein; HOMA: homeostatic model assessment; TC: total cholesterol; TG: triglycerides; SBP: systolic blood pressure; WC: waist circumference.

Design and study population

The Active Smarter Kids (ASK) study was a seven-month cluster-randomized controlled trial conducted in November 2014 to June 2015 in western Norway (23). Sixty invited schools (including at least seven children in each class) were randomized at a 1:1 ratio by a neutral third party. After randomization, three schools (two intervention schools and one control school), withdrew from participation. In total, 1145 out of 1175 invited 5th graders from 57 schools agreed to participate, whereof valid data were obtained from 1129 children. The ASK study design, sample procedures and methods are described in detail elsewhere (23) and will only briefly be presented herein.

Intervention

The PA intervention consisted of three components, consisting of 165 min of additional PA per week for the intervention children compared to the control children: 1) physically active academic lessons executed in the playground (3 x 30 min each week), 2) PA breaks during classroom lessons (5 min each school day), and 3) PA homework prepared by the teachers (10 min each school day). The intervention was a mandatory part of the school curriculum in intervention schools, adding to the existing mandatory curriculum-prescribed PA (45 min per week) and physical education (PE) (90 min per week), amounting to a total PA level of 300 min per week (60 min per day) of PA/PE. Control schools were encouraged only to provide the mandatory amount of PA/PE (135 min per week).

The intervention was delivered by the fifth grade classroom teachers. Three comprehensive pre-intervention seminars and two regional refreshing meetings during the intervention period were conducted to empower, support and qualify the teachers to deliver the intervention. We aimed to provide activities that were inclusive and varied to allow unfit or unenthusiastic children to experience mastery and enjoyment. Approximately 25% of the daily PA in school was intended to be of vigorous intensity; the children should be "*sweating and out of breath*".

Adherence to the protocol

On a monthly basis, school teachers reported the weekly amount of PA (duration in minutes and intensity). Intensity of PA was defined as 1 = low intensity, 2 = moderate intensity, and 3 = vigorous intensity. Intervention schoolteachers reported for all three components of the intervention (PA educational lessons, PA breaks, and PA homework).

Physical activity

Physical activity was measured by the ActiGraph GT3X+ accelerometer (ActiGraph GT3X+, LLC, Pensacola, Florida, USA). Children were instructed to wear the accelerometer on the right hip at all

times over seven consecutive days, except during water-based activities, showering, orsleeping. Valid monitor wear-time was defined as reaching ≥480 min per day between the hours of 6:00 and 24:00 and a valid school-day as reaching ≥180 min per day between 9:00 and 14:00. Periods of ≥20 min of zero counts were defined as non-wear time (24). Children having valid wear-time in ≥4 (out of 7) days and in ≥3 (out of 5) school days were included in the analysis. Previously established and validated cut-off points were used to define sedentary time (<100 counts per min (cpm)), moderate-to-vigorous-intensity PA (MVPA) (>2296 cpm) and VPA (>4012 cpm) (25,26). KineSoft analytical software (KineSoft version 3.3.80, Loughborough, UK) was used to analyze all accelerometer data. Accelerometer data were collected at baseline (April-June 2014), midway through the intervention (January-February 2015) and at follow-up (April-June 2015).

Cardiorespiratory fitness

Cardiorespiratory fitness was measured using the validated Andersen shuttle-run test (27,28). Children ran 20 meters between two lines in an intermittent pattern, touching the floor with one hand behind the line at each turn. They ran for 15 secs and stood still for another 15 sec. The test lasted for 10 min, where the total distance (meters) covered was used as an expression of CRF in the analyses. The performance in the Andersen test was converted into VO_{2peak} by the following equation; boys VO_{2peak} = 27.1689 + (0.0397 × distance (m)) – (0.1698 × body mass (kg)), girls VO_{2peak} = 32.5793 + (0.0309 × distance (m)) – (0.2351 × body mass (kg)) (29).

Anthropometry

BMI

Body mass was measured to the nearest 0.1 kg using an electronic scale (Seca 899, SECA GmbH, Hamburg, Germany) with children wearing light clothing or underwear (preferred) depending on the acceptance of the child. A portable Seca 217 (SECA GmbH, Hamburg, Germany) was used to measure stature to the nearest 0.1 cm with the barefooted child facing forward. Body mass index (BMI; $kg*m^{-2}$) was calculated as weight (kg) divided by the height squared (m^2).

Waist circumference

Waist circumference (WC) was measured using an ergonomic circumference measuring tape, Seca 201 (SECA GmbH, Hamburg, Germany). With the child's abdomen relaxed at the end of a gentle expiration, two measures were taken between the lowest rib and the iliac crest to the nearest 0.5 cm. If the measures differed by >1 cm, we obtained a new measurement. The mean of the two closest measurements (≤ 1 cm) was used for analyses.

Blood sampling

A phlebotomist or nurse collected intravenous blood samples from the children's antecubital veins after overnight fast. Blood samples and serum were analyzed by an ISO-certificated laboratory for traditional risk factors related to cardiometabolic diseases, such as insulin, glucose, triglycerides (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and total cholesterol (TC). The TC:HDL-ratio was calculated to represent dyslipidemia. Insulin resistance was defined by the homeostatic model assessment (HOMA)-score = [insulin (pmol/L)*glucose (mmol/L)]/135 (30).

Resting blood pressure

Systolic (SBP) and diastolic (DBP) blood pressure was measured by the Omron HEM-907 automated BP monitor (Omron Healthcare, Inc, Vernon Hills, IL, US). The device was previously validated according to the AAMI validation protocol (31) and to the validation criteria of the international protocol for BP measuring devices (32). The children were measured in a quiet room after resting for 10 min in a seated position (without distractions) on the upper right arm. Four measurements were taken with one-min pauses in between, and the mean of the last three measurements was used for analysis. If the measurements differed by more than 5 mmHg, a new measurement was taken, and the mean of the last four blood pressure measurements was used.

Maturity

Children self-assessed their puberty stage according to the Tanner scale (33) using color pictures as proposed by Carel and Leger (34). We created a relaxed atmosphere for this assessment, and the children were given the standardized series of images with explanatory text in a private room. We used breast and genital development for girls and boys, respectively, as a measure of pubertal stage. Category 1: prepubertal stage; category 2: first signs of puberty; categories 3-5: collapsed due to a small number of children representing more advanced pubertal stages than category 2, with category 5 being fully mature.

Demographic characteristics

We obtained self-reported educational level from the children's parents/legal guardians to assess socioeconomic status (SES). Education level was categorized into three groups using the highest educational level obtained by the mother or the father: i) upper or lower secondary school, ii) university < four years, and iii) university ≥ four years.

Ethics

The study protocol was approved by the South-East Regional Committee for Medical Research Ethics (2013/1893), and all procedures and methods adhered to the ethical guidelines of the World Medical Association's Declaration of Helsinki and its subsequent revisions (35). Prior to commencement of the investigation, written informed consent was obtained from each child's parent(s) or guardian(s). The ASK study is registered in the Clinicaltrials.gov registry [NCT02132494].

Statistics

All values exceeding five standard deviations from the mean were excluded before analysis. A continuous clustered risk score was calculated as the mean age-standardized z-scores of WC, SBP, TC:HDL-ratio, TG, HOMA-score, and CRF (inversed). The residuals of change between baseline and follow-up for the cardiometabolic risk factors were normally distributed, although some of the single cardiometabolic risk factors were skewed, and the respective risk variables were therefore not transformed.

Descriptive statistics are presented as the mean and standard deviation (SD), median and interquartile range (IQR), or frequency (%). Three analyses were used to evaluate the effect of the intervention, all conducted according to the statistical analysis plan (23) and by using linear mixed models with school as a random effect to account for the cluster effect: 1) The effect of the intervention was investigated using intention-to-treat analysis, including all children who had valid data on the single cardiometabolic risk factors at both baseline and follow-up. Multiple imputations were also conducted for missing data, imputed from relevant variables using a Markov Chain Monte Carlo procedure. We assumed data were missing at random (36). The models included group and baseline values as independent variables and change as the dependent variable. 2) Per-protocol analysis was conducted using similar models but only included data from intervention schools reporting ≥ 80% of prescribed PA and control schools reporting < 120% of the curriculum-prescribed PA (135 min/week). 3) In line with similar studies (22,37), we tested the moderating effects of cardiometabolic baseline values and sex. We hypothesized that intervention effects would be larger in girls, as they typically have lower levels of MVPA (38) and CRF (39). The subgroup analyses were performed on children having valid measures in all cardiometabolic risk variables (n= 769) by including the following interaction terms in three separate analysis: a) group*tertile (tertiles were defined by baseline values in the clustered cardiometabolic risk score), b) group*sex, and c) group*sex*tertile. All models were full factorial models; two-way interaction models included main effects, and three-way interaction models included main effects and two-way interactions. The interaction effect by group*tertile (model a) and group*sex*tertile (model c) was investigated in linear mixed models using group, subgroup(s), and the interaction term as independent variables and

change as the dependent variable. The moderating effect of group*sex (model b) also included baseline values of the single cardiometabolic risk factor as an independent variable in the respective models.

Analyses were performed using actual units and using z-scores to allow for a meaningful interpretation of results. All analyses were conducted using IBM SPSS version 25 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). A p-value < 0.05 was considered statistically significant in analysis of the main effects, whereas a p-value < 0.1 was applied to indicate statistical significance of interaction terms (40).

Results

All schools were represented in the analysis (except in the per-protocol analysis). Some children did not provide data at either baseline or follow-up and were excluded from analysis as follows: WC (n=8), SBP (n=10), TC:HDL-ratio, TG and HOMA (n=82 in each variable), CRF (n=19), and clustered risk score (n=99). Seven of the children dropped out during the intervention period (see flowchart in Fig. 1).

Table 1 shows the baseline characteristics of the intervention and control groups. In addition, Table A.1 (appendix) shows the baseline characteristics of PA levels and sedentary time for girls and boys separately.

Table 1 Baseline characteristics of intervention and control school children

		Intervention		**Control**
	N	Mean (±SD)/ median [Q1-Q3]/ n (%)	N	Mean (±SD)/ median [Q1-Q3]/ n (%)
Age (yr)	596	10.2 (0.3)	533	10.2 (0.3)
Height (cm)	579	142.6 (6.8)	517	142.8 (6.8)
Weight (kg)	578	36.9 (8.0)	517	37.2 (8.1)
Puberty (Tanner)	569		512	
Stage 1		170 (30)		139 (27)
Stage 2		330 (58)		318 (62)
Stage 3-5		69 (12)		55 (11)
Parents' education level	578		491	
≤ Upper secondary school		179 (31)		172 (35)
<4 years of university		179 (31)		142 (29)
≥4 years of university		220 (38)		177 (36)
Physical activity (school day)	538		538	
Total PA (cpm)		650 (186)		641 (192)
MVPA (min/day)		29 (11)		28 (10)
SED (min/day)		179 (20)		179 (21)
Physical activity (full day)	542		464	
Total PA (cpm)		745 (299)		723 (257)
MVPA (min/day)		77 (28)		74 (24)
SED (min/day)		468 (57)		468 (60)
% achieving PA guidelines		71		70
Cardiometabolic risk factors				
BMI (kg/m²)	578	17.5 [15.9-19.5]	517	17.2 [16.0-19.6]
WC (cm)	577	60.3 [56.0-65.5]	517	60.3 [57.0-65.7]
SBP (mm Hg)	575	105.5 (8.3)	511	105.2 (8.7)
DBP (mm Hg)	575	58.2 (5.8)	511	57.5 (6.7)
LDL cholesterol (mmol/L)	532	2.5 (0.7)	471	2.5 (0.6)
HDL cholesterol (mmol/L)	533	1.6 (0.3)	471	1.6 (0.3)
Total cholesterol (mmol/L)	533	4.4 (0.7)	471	4.5 (0.7)
TC:HDL-ratio	533	2.8 [2.4-3.3]	471	2.8 [2.4-3.2]
Triglyceride (mmol/L)	533	0.69 [0.55-0.89]	471	0.68 [0.55-0.89]
Glucose (mmol/L)	533	5.0 (0.3)	471	5.0 (0.3)
Insulin (pmol/L)	532	49.5 [35.3-68.2]	470	49.2 [35.1-66.8]
HOMA-score	532	1.8 [1.3-2.5]	470	1.8 [1.3-2.5]
Andersen (m)	550	893.6 (102.6)	495	891.9 (103.7)
Estimated VO_{2peak} (mL/kg/min)	549	54.2 (5.5)	495	54.0 (5.6)
Clustered risk score*	484	0.004 (0.66)	429	-0.005 (0.63)

Abbreviations BMI: body mass index; cpm: counts per min; CRF: cardiorespiratory fitness; DBP: diastolic blood pressure; HDL: high-density lipoprotein; HOMA: homeostatic model assessment; LDL: low-density lipoprotein; MVPA; moderate-to-vigorous physical activity; PA: physical activity: SBP: systolic blood pressure; SED: sedentary; TG: triglyceride. Risk variables in *italics* were skewed (nonnormal distribution). Values for PA were adjusted for valid wear time. PA guidelines represent children who achieved a mean minimum of 60 min/day of MVPA. *The clustered risk score was based on the following six cardiometabolic risk variables: WC, SBP, TC:HDL-ratio, TG, HOMA-score, and CRF (inversed).

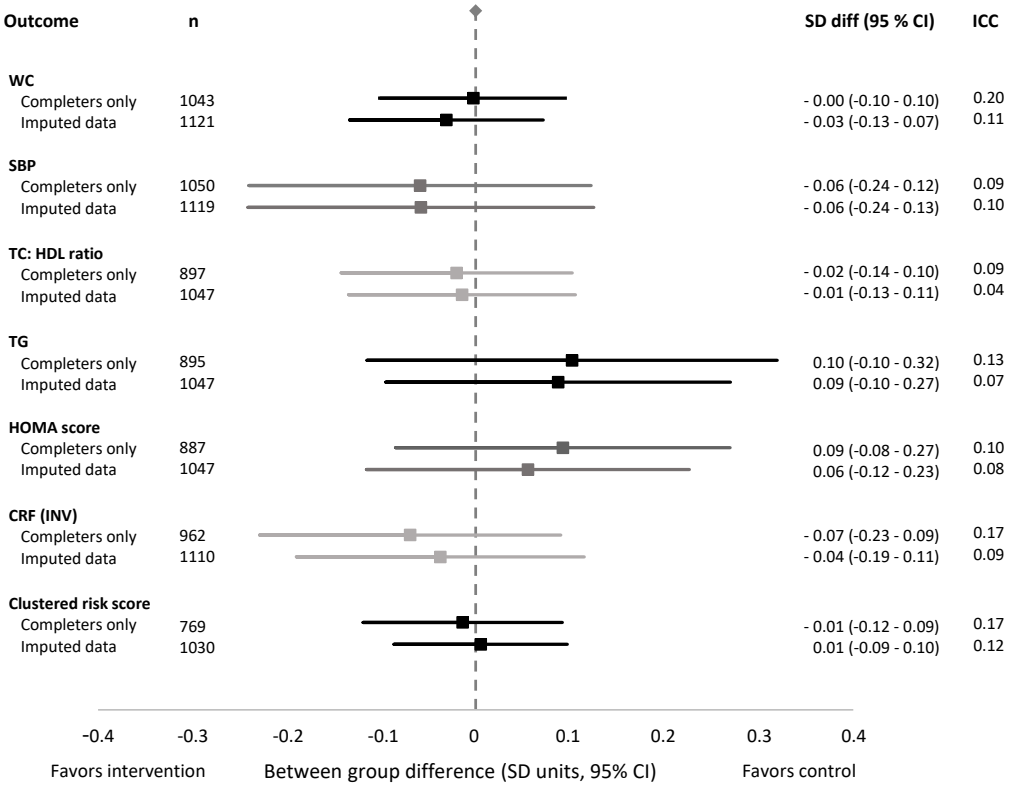

Fig. 2 The intervention effect (intention-to-treat analysis) from completers only (n = 769-1050) and from imputed data (N = 1030-1121) for those children having a valid measure at either baseline or follow-up. Effect sizes are presented in standardized units (SD) and 95% confidence interval. Cardiorespiratory fitness was inversed (CRF*-1).
95% CI: 95% confidence interval; HDL: high-density lipoprotein; HOMA: homeostatic model assessment; ICC: intraclass correlation coefficient (school); INV: inverse; SD: standardized difference; SBP: systolic blood pressure; TC: total cholesterol; TG: triglyceride; WC: waist circumference.

No significant intervention effects were found for any of the six cardiometabolic risk factors or the clustered risk score in either the intention-to-treat (Fig. 2) or the per-protocol analysis (Table 2). The effect sizes were very small in the intention-to-treat analyses for completers only (- 0.07 to - 0.01 SD units), for the imputed data (- 0.06 to - 0.01 SD units), and the per-protocol analysis (- 0.05 to - 0.001 SD units).

Table 2 Effects of the intervention on cardiometabolic risk factors (per-protocol analysis)

	n	Group difference, β (95% CI)	*p*-value	ICC
WC	831	0.01 (-0.85 to 0.87)	0.98	0.19
SBP	833	-0.23 (-2.00 to 1.53)	0.79	0.09
TC:HDL-ratio	727	-0.02 (-0.11 to 0.08)	0.71	0.08
TG	727	0.03 (-0.03 to 0.10)	0.32	0.10
HOMA-score	718	0.14 (-0.14 to 0.42)	0.32	0.11
CRF	769	- 0.05 (-17.71 to 17.62)	0.97	0.14
Clustered risk score	624	-0.003 (-0.11 to 0.10)	0.95	0.14

Abbreviations: 95% CI: 95% confidence interval; β: beta-coefficient; CRF: cardiorespiratory fitness; HDL: high-density lipoprotein; HOMA: homeostatic model assessment; ICC: intraclass correlation coefficient (school); TC: total cholesterol; TG: triglyceride; SBP: systolic blood pressure; WC: waist circumference. Per-protocol: intervention schools reporting ≥ 80% of prescribed PA and control schools reporting < 120% of the curriculum prescribed PA.

Fig. 3 shows the difference in change in each cardiometabolic risk factor between the intervention and control schools by tertiles (group*tertile), representing subgroups of children having the least favorable baseline values of clustered cardiometabolic risk factors in tertile 1 and the most favorable baseline values in tertile 3. A significant moderating effect by tertile was found for SBP (p for interaction = 0.07), TC:HDL-ratio (p for interaction = 0.03) and the clustered cardiometabolic risk score (*p* for interaction = 0.01).

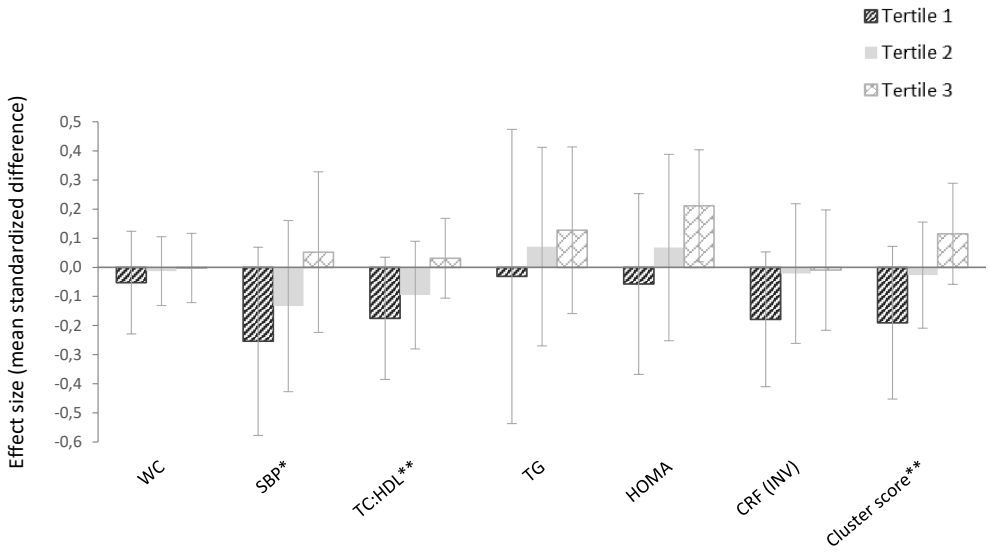

Fig. 3 Subgroup differences between the intervention and control group by tertiles (tertile 1 is the least favorable group and tertile 3 is the most favorable group in the clustered risk score at baseline). Effect sizes are presented in standardized units (SD) ± 95% confidence interval (CI). The subgroup (n= 769) of children had valid measures in all cardiometabolic risk variables. Cardiorespiratory fitness was inversed (CRF*-1). The clustered risk score consisted of the following variables; WC, SBP, TC:HDL-ratio, TG, HOMA-score and CRF (inversed).
* Significant group*tertile interaction ($p < 0.1$). ** Significant group*tertile interaction ($p < 0.05$).

The moderating effect of sex on the change in each cardiometabolic risk factor is shown in Fig. 4. A significant moderating effect for group*sex was found for WC (*p* for interaction = 0.03) and CRF (*p* for interaction <0.001) in favor of the girls. In the stratified analysis, only CRF in girls reached significance and showed that CRF increased more from baseline to follow-up in girls from intervention schools (0.19 SD units) compared to girls from control schools.

No significant three-way interaction effects were found for any cardiometabolic risk factor or for the clustered risk score (*p* for interaction ≥ 0.25).

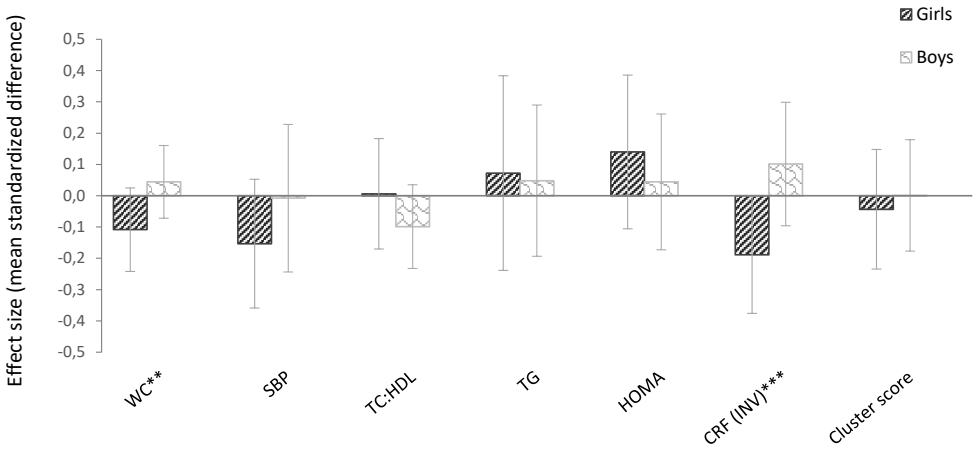

Fig. 4 Subgroup differences between the intervention and control groups by sex. Effect sizes are presented in standardized units (SD) ± 95% confidence interval (CI). The subgroup (n= 769) of children had valid measures in all cardiometabolic risk variables. Cardiorespiratory fitness was inversed (CRF*-1). The clustered risk score was based on the following variables: WC, SBP, TC:HDL-ratio, TG, HOMA-score and CRF (inversed).
* Significant group*sex interaction ($p < 0.1$). ** Significant group*sex interaction ($p < 0.05$). *** Significant group*sex interaction ($p < 0.001$)

Discussion

We found no significant intervention effect of the ASK school-based PA intervention on any of the cardiometabolic risk factors or the clustered risk score in either the intention-to-treat or the per-protocol analysis in the total population. We did, however, find significant moderation by baseline cardiometabolic risk on SBP, TC:HDL-ratio and the clustered cardiometabolic risk score. Moreover, we found moderation by sex, showing that girls had more favorable effects on WC and CRF compared to boys.

There were no significant intervention effects on children's PA or sedentary time in the total sample from baseline to follow-up, measured objectively by accelerometers (41). High levels of PA in the control group may explain the lack of effect, which is not an unusual observation in PA intervention trials (42). Furthermore, the high baseline levels of PA in the ASK study could indicate a potential ceiling effect in most children. ASK children from both the intervention schools and control schools were, on average, more physically active than population-based samples of 9-10-year-old Norwegian children (43) as well as European and US children (38). Since the premise in the ASK study was that increased PA would cause a change in cardiometabolic risk factors (including CRF), this is likely to be the main reason why we were unable to detect measurable benefits on any of the cardiometabolic risk factors. Another reason could also be that the intervention potential, with respect to the cardiometabolic risk factors, was relatively low, as children have generally healthy cardiometabolic risk profiles (44). Furthermore, we found no difference in cardiometabolic risk factor levels at follow-up between children from intervention and control schools in the per-protocol analysis. The per-protocol analysis excluded only one intervention school, while 13 control schools were excluded. It seems plausible that overestimation of PA levels from intervention school teachers could have occurred, since no difference was found in accelerometer data (41).

Children with the most unfavorable baseline values in the clustered risk score (tertile 1) benefitted more from the PA intervention than children with more favorable values. Although no stratified analyses of the intervention effect by tertiles reached statistical significance, there was a consistent pattern of mean effect sizes in favor of the children in tertile 1, supporting findings from a previous school-based PA intervention study (22). Post hoc analysis investigating accelerometer data measured midway through the ASK intervention period showed a significant moderating effect by tertile on sedentary time during all day, and in the stratified analysis, children in tertile 1 were significantly less sedentary than their peers from control schools (appendix, Fig. A.1). No other midway analysis reached significance, but the all-day PA levels reflect the consistent pattern observed of changes in favor of the group of children having the least favorable baseline

cardiometabolic risk profile. In addition, when comparing cardiometabolic risk factor levels with international age- and sex specific reference values (39), tertile 1 had a mean standardized difference in the clustered risk score of 0.52 SD (95% CI 0.47 to 0.58). Although less favorable, the clustered risk score was only moderately higher than the international reference values, which could indicate that children in the ASK study, even with the most unfavorable risk profile, were within healthy cardiometabolic ranges and had a relatively low potential to change.

The subgroup analysis revealed significant interaction effects by sex on WC and CRF, and girls from the intervention schools increased their CRF levels significantly more between baseline and follow-up than girls from control schools. Since girls' CRF levels have been shown to stagnate or even decrease after the age of nine (45), this is a positive outcome of the intervention. Sex-specific differences on outcomes in school-based PA interventions have been a controversial issue. However, a recent review of the effect of school-based RCT PA interventions on CRF showed a significant increase in CRF among girls but not among boys (21). To better understand the different responses in girls and boys observed in the present study, we performed post hoc analysis of the midway accelerometer data (appendix, Fig. A.2). The analysis showed significant interaction effects by sex on total PA, MVPA and VPA both during school and all day in favor of the girls. Compared to girls from control schools, girls from intervention schools were significantly more physically active in total, did more VPA and were less sedentary during school hours. In accordance with studies showing that boys engage in more PA and are less sedentary than girls (38), girls at baseline in the ASK study had significantly lower total PA levels, were less in MVPA and VPA, and were more sedentary both during school and all day than the boys (appendix, Table A.1). Furthermore, girls had lower levels of CRF and in general a less favorable risk profile than boys at baseline (44). Thus, the girls had a larger potential for change as a response to the PA intervention than the boys, which plausibly can explain the positive outcomes.

PA interventions in school children have shown conflicting results with respect to effects on cardiometabolic risk factors, although with some indications of higher success in improving fitness levels (18-21) rather than the *traditional* cardiometabolic risk factors such as adiposity, blood pressure, glucose intolerance, and dyslipidemia (18,19). Exploring intervention effects in generally healthy young children could, however, underestimate the true intervention effect. Thus, future studies investigating intervention effects on cardiometabolic risk factors in children could explore subgroups of children with the most unfavorable risk profile at baseline, including sex specific analysis, as done in the present study.

The cluster-RCT design is a major strength of the study. The intervention was delivered by the classroom teachers, which increases the external validity of the study. Other strengths are the objectively measured PA, the relatively large sample size and low attrition rate.

The results should be generalized with caution, as the sample only represents one county in Norway. The ASK intervention was designed to first and foremost affect the main outcome, academic performance, and the daily dose of PA during a limited intervention period of 7 months may have been insufficient to positively affect cardiometabolic risk factors. Furthermore, the weekly PA reports were completed by the school teachers, which might have introduced subjective errors in the per-protocol analysis. Other limitations include a generally low intervention potential due to initial high PA levels and that most children already fulfilled the guidelines of at least 60 min of MVPA daily. The ASK study did not record food intake, but the randomized design will have countered any confounding effect of diet.

Conclusion

The present study found no significant intervention effect of active academic classes and short active breaks during school hours on children's cardiometabolic health in the overall sample. However, subgroup analysis showed that girls had more favorable effects on WC and CRF than boys, and the data indicate that PA interventions in school can contribute to better cardiometabolic health in those subgroups of children that have the most unfavorable cardiometabolic risk profiles at baseline.

Acknowledgments

The authors are grateful to the children and their families, principals and teachers from the 57 participating schools involved in the ASK study for their daily support, time and effort during the study. We would also like to thank the personnel involved in the relatively comprehensive data collection. Finally, we are thankful for the financial support from the Research Council of Norway (grant number 221047/F40), Western Norway University of Applied Science, the Norwegian School of Sports Science and the Gjensidige Foundation (grant number 1042294), without which this study would not have been possible.

References

1. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650-6.
2. Berenson GS, Wattigney WA, Tracy RE, et al. Atherosclerosis of the aorta and coronary arteries and cardiovascular risk factors in persons aged 6 to 30 years and studied at necropsy (The Bogalusa Heart Study). Am J Cardiol 1992;70:851-8.
3. Strong JP, Malcom GT, McMahan CA, et al. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA 1999;281:727-35.
4. WHO. World Health Organization. Prevention and control of noncommunicable diseases: implementation of the global strategy. Geneva: WHO; 2008.
5. Strong WB, Malina RM, Blimkie CJ, et al. Evidence based physical activity for school-age youth. J Pediatr 2005;146:732-7.
6. Kristensen PL, Moeller NC, Korsholm L, et al. The association between aerobic fitness and physical activity in children and adolescents: the European youth heart study. Eur J Appl Physiol 2010;110:267-75.
7. Ortega FB, Ruiz JR, Castillo MJ, Sjostrom M. Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes (Lond) 2008;32:1-11.
8. Katzmarzyk PT, Church TS, Blair SN. Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men. Archives of Internal Medicine 2004;164:1092-7.
9. Blair SN, Kampert JB, Kohl HW, 3rd, et al. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. JAMA 1996;276:205-10.
10. Hallal PC, Andersen LB, Bull FC, et al. Global physical activity levels: surveillance progress, pitfalls, and prospects. Lancet 2012;380:247-57.
11. Dalene KE, Anderssen SA, Andersen LB, et al. Secular and longitudinal physical activity changes in population-based samples of children and adolescents. Scand J Med Sci Sports 2018;28:161-71.
12. Farooq MA, Parkinson KN, Adamson AJ, et al. Timing of the decline in physical activity in childhood and adolescence: Gateshead Millennium Cohort Study. Br J Sports Med 2018;52:1002-6.
13. Malina RM. Physical activity and fitness: pathways from childhood to adulthood. Am J Hum Biol 2001;13:162-72.
14. Raitakari OT, Porkka KV, Taimela S, Telama R, Rasanen L, Viikari JS. Effects of persistent physical activity and inactivity on coronary risk factors in children and young adults. The Cardiovascular Risk in Young Finns Study. Am J Epidemiol 1994;140:195-205.
15. Story M, Nanney MS, Schwartz MB. Schools and obesity prevention: creating school environments and policies to promote healthy eating and physical activity. Milbank Q 2009;87:71-100.
16. (WHO). WHO. Global Strategy on Diet, Physical Activity and Health. http://www.who.int/nmh/wha/59/dpas/en/2004.
17. Naylor PJ, McKay HA. Prevention in the first place: schools a setting for action on physical inactivity. Br J Sports Med 2009;43:10-3.
18. Sun C, Pezic A, Tikellis G, et al. Effects of school-based interventions for direct delivery of physical activity on fitness and cardiometabolic markers in children and adolescents: a systematic review of randomized controlled trials. Obes Rev 2013.
19. Dobbins M, Husson H, DeCorby K, LaRocca RL. School-based physical activity programs for promoting physical activity and fitness in children and adolescents aged 6 to 18. Cochrane Database Syst Rev 2013;2:CD007651.

20. Kriemler S, Meyer U, Martin E, van Sluijs EM, Andersen LB, Martin BW. Effect of school-based interventions on physical activity and fitness in children and adolescents: a review of reviews and systematic update. Br J Sports Med 2011;45:923-30.
21. Pozuelo-Carrascosa DP, Garcia-Hermoso A, Alvarez-Bueno C, Sanchez-Lopez M, Martinez-Vizcaino V. Effectiveness of school-based physical activity programmes on cardiorespiratory fitness in children: a meta-analysis of randomised controlled trials. Br J Sports Med 2017.
22. Resaland GK, Aadland E, Nilsen AKO, Bartholomew JB, Andersen LB, Anderssen SA. The effect of a two-year school-based daily physical activity intervention on a clustered CVD risk factor score-The Sogndal school-intervention study. Scand J Med Sci Sports 2018;28:1027-35.
23. Resaland GK, Moe VF, Aadland E, et al. Active Smarter Kids (ASK): Rationale and design of a cluster-randomized controlled trial investigating the effects of daily physical activity on children's academic performance and risk factors for non-communicable diseases. BMC Public Health 2015;15:709.
24. Esliger DW, Copeland JL, Barnes JD, Tremblay MS. Standardizing and Optimizing the Use of Accelerometer Data for Free-Living Physical Activity Monitoring. Journal of Physical Activity & Health 2005;2:366-83.
25. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. J Sport Sci 2008;26:1557-65.
26. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of Accelerometer Cut Points for Predicting Activity Intensity in Youth. Med Sci Sport Exer 2011;43:1360-8.
27. Andersen LB, Andersen TE, Andersen E, Anderssen SA. An intermittent running test to estimate maximal oxygen uptake: the Andersen test. J Sports Med Phys Fitness 2008;48:434-7.
28. Aadland E, Terum T, Mamen A, Andersen LB, Resaland GK. The Andersen aerobic fitness test: reliability and validity in 10-year-old children. PLoS One 2014;9:e110492.
29. Aadland E, Andersen LB, Lerum O, Resaland GK. The Andersen aerobic fitness test: New peak oxygen consumption prediction equations in 10 and 16-year olds. Scand J Med Sci Sports 2017.
30. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
31. White WB, Anwar YA. Evaluation of the overall efficacy of the Omron office digital blood pressure HEM-907 monitor in adults. Blood Press Monit 2001;6:107-10.
32. El Assaad MA, Topouchian JA, Darne BM, Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit 2002;7:237-41.
33. Tanner JM. Growth at adolescence; with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity. 2d ed. Springfield, Ill.1962.
34. Carel JC, Leger J. Precocious puberty. New England Journal of Medicine 2008;358:2366-77.
35. Assoc WM. World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Jama-J Am Med Assoc 2013;310:2191-4.
36. Little RJ, D'Agostino R, Cohen ML, et al. The Prevention and Treatment of Missing Data in Clinical Trials. New England Journal of Medicine 2012;367:1355-60.
37. Resaland GK, Moe VF, Bartholomew JB, et al. Gender-specific effects of physical activity on children's academic performance: The Active Smarter Kids cluster randomized controlled trial. Prev Med 2018;106:171-6.
38. Cooper AR, Goodman A, Page AS, et al. Objectively measured physical activity and sedentary time in youth: the International children's accelerometry database (ICAD). International Journal of Behavioral Nutrition and Physical Activity 2015;12.
39. Ruiz JR, Cavero-Redondo I, Ortega FB, Welk GJ, Andersen LB, Martinez-Vizcaino V. Cardiorespiratory fitness cut points to avoid cardiovascular disease risk in children and adolescents; what level of fitness should raise a red flag? A systematic review and meta-analysis. Br J Sports Med 2016.

40. Twisk JWR. Applied Multilevel Analysis. A Practical Guide. Cambridge, UK: Cambridge University Press; 2006.
41. Resaland GK, Aadland E, Moe VF, et al. Effects of physical activity on schoolchildren's academic performance: The Active Smarter Kids (ASK) cluster-randomized controlled trial. Prev Med 2016;91:322-8.
42. Waters L, Reeves M, Fjeldsoe B, Eakin E. Control group improvements in physical activity intervention trials and possible explanatory factors: a systematic review. J Phys Act Health 2012;9:884-95.
43. Health TNDo. Physical activity in Norwegian 6, 9 and 15-year olds.
44. Stavnsbo M, Skrede T, Aadland E, et al. Cardiometabolic risk factor levels in Norwegian children compared to international reference values: The ASK study. Unpublished results - *In review* 2019.
45. Stavnsbo M, Resaland GK, Anderssen SA, et al. Reference values for cardiometabolic risk scores in children and adolescents – suggesting a common standard. Atherosclerosis 2018;278:299-306.

APPENDIX I

Approval letter from the Regional Committees for Medical and Health Research Ethics

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Anette Solli Karlsen	22845522	04.03.2014	2013/1893/REK sør-øst A
			Deres dato:	**Deres referanse:**
			28.01.2014	

Vår referanse må oppgis ved alle henvendelser

Sigmund Anderssen
Høgskulen i Sogn og Fjordane

2013/1893 ASK - Active Smarter Kids

Forskningsansvarlig: Høgskulen i Sogn og Fjordane
Prosjektleder: Sigmund Anderssen

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 13.02.2014. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikklovens § 4.

Opprinnelig prosjektbeskrivelse
Målsettingen i dette prosjektet er å undersøke effekten av en time daglig fysisk aktivitet i skolehverdagen for elever i femte klasse.
En eventuell effekt skal måles på skoleprestasjoner i matematikk, lesing og engelsk, på kognitive prestasjoner og på helsevariabler som lipider og hjernederivert nevrotrofisk faktor (Brain Derived Neurotrophic Factor, BDNF), som påvirker hjernecellers utvikling og funksjon.
Prosjektet har et klynge randomisert design. Skolen er enheten med to grupper, en intervensjons- og en kontrollgruppe. Forsøket har en varighet på åtte måneder. I alt 1196 barn som går i femte klasse i ulike skoler i Sogn og Fjordane skal spørres om deltakelse. Halvparten av skoleklassene vil bli randomisert til intervensjonsgruppen med daglig fysisk aktivitet, mens den andre halvdelen vil komme i kontrollgruppen og får fysisk aktivitet som vanlig i skolen, dvs. to timer per uke. Den fysiske aktiviteten, som intervensjonsgruppen tilbys er variert, og etter endt forsøk, vil kontrollgruppen bli tilbudt den sammen intervensjonen dvs. når de går i 6. klasse. Med et slikt design vil alle få det samme tilbudet.
Hele utvalget vil undersøkes ved baseline og etter åtte måneder med en rekke fysiske tester, med antropometriske mål, høyde, vekt midjemål og hudtykkelse, med blodtrykk, flere kognitive tester, spørreskjema om livskvalitet, kosthold, samt vil det bli tatt blodprøver for å måle lipidmønster i blod, glukose og BDNF.
Det er utarbeidet et informasjonsskriv med samtykkeerklæring som er adressert både til foreldrene og til barna. Noen av deltakerne, dvs. barn og lærere, vil bli spurt om å delta i en kvalitativ studie, hvor intervju skal tas opp på bånd, transskriberes og analyses. I denne kvalitative delen av studien vil man også benytte seg av fotografi, dvs. man ønsker å ta bilder i de fysiske aktivitetene i prosjektet, og disse vil bli forelagt deltakerne og brukt i intervjusituasjonen.

Saksbehandling
Søknaden ble behandlet i møte 24.10.2013, og det ble fattet et utsettende vedtak. Komiteen ba om tilbakemelding på følgende punkter:

1. Datamaterialet vil bli anonymisert for forskerne i prosjektet 31.12 2016, men en navneliste vil bli

oppbevart hos en tredje person, dvs. hos NSD. Man opplyser også i informasjonsskrivet at man planlegger å be barna nå de er fylt 16 år om deres samtykke til å anvende data for senere forskning. Hva denne forskningen vil medføre står det ingenting om, og det går heller ikke klart fra prosjektprotokollen hva som planlegges. Prosjektbeskrivelsen omtaler ikke en slik eventuell oppfølging.
2. I informasjonsskrivet ber man om at data fra undersøkelsen kan kobles mot nasjonalt helseregister, medisinsk fødselsregister og mor/barn-registeret. Denne koblingen er ikke begrunnet noe sted, og man kan heller ikke i prosjektbeskrivelsen finne noen omtale av en slik kobling som man ber deltakerne samtykke til i informasjonsskrivet.
3. Det fins ingen opplysninger i informasjonsskrivet om den kvalitative delen av studien og heller ingen informasjon til lærerne som vil bli bedt om å delta i den delen av studien er vedlagt.
4. Prosjektledelsen har på side 8 i søknadsskjemaet diskutert ulike mulig ulemper som prosjektet kan ha på barna og argumentere for at prosjektet ikke kan ha slike ulemper som de diskuterer. En mulig ulempe er muligens uteglemt i diskusjonen og det er relatert til gruppepress. Hva med elever som ikke vil delta, for eksempel en elev i en klasse på 20 som ikke vil være med. Om hele klassen er randomisert til 1 times fysisk aktivitet hver dag, hva skjer med den ene elevens undervisningstilbud og hva kan han/hun eventuelt utsette for av mobbing/gruppepress? Det savnes en diskusjon av dette aspektet og hvordan man skal ivareta «ikke-deltakere».
5. Komiteen ber om en nærmere redegjørelse om behovet for en beredskap i forbindelse med informasjon som kan komme opp som resultat av prosjektet. Kan det tenkes uventede funn i analysene av blodprøver? Kan det tenkes svar på spørsmål i spørreskjemaet som kan tyde på det trenges en eller annen form for oppfølging?
6. Norsk versjon engelsk spørreskjema må ettersendes.

Prosjektleder har sendt tilbakemelding, denne ble mottatt 28.01.2014.

Om komiteens merknader fremkommer det av tilbakemeldingen:

1. Det kan i fremtiden være aktuelt å se på langtidseffektene av intervensjonen. Kontrolldeltakerne vil bli tilbudt samme intervensjon som studiegruppen, noe som i første omgang vil vanskeliggjøre en sammenligning mellom gruppene. Av denne grunn omfatter ikke protokollen en oppfølging på det nåværende tidspunkt. I midlertid vil en oppfølging av deltakerne i et longitudinelt design muliggjøre en evaluering av langtidseffekter, og for å sikre at man kan be barna om deltakelse i et slikt eventuelt oppfølgingsstudie ønsker man nå å legge dette inn i informasjonsskrivet. Formuleringene i informasjonsskrivet er endret slik at dersom barnet planlegges undersøkt på nytt eller dersom data vil bli benyttet etter barna er fylt 16 år, så vil man be om et nytt samtykke for dette.
2. Det skal innhentes data fra medisinsk fødselsregister og MoBa-registeret, og disse koblingene er nå spesifisert i informasjonsskrivet.
3. Det foreligger nå en beskrivelse av den kvalitative delen av prosjektet, og det er utformet separate informasjonsskriv for deltakerne i denne delen.
4. Randomiseringen til intervensjon eller kontroll vil foregå på skolenivå, og ved intervensjonsskolene vil den ekstra timen med fysisk aktivitet inngå som en ordinær del av det pedagogiske tilbudet. Det vil derfor ikke oppleves som press på enkeltelever i forhold til deltakelse i prosjektet eller ikke. For de elever som av ulike årsaker søker fritak fra fysisk aktivitet, vil skolen på ordinær måte finne andre undervisningstilbud.
5. Eventuelle funn som måtte avdekkes ved deltakelse i prosjektet vil håndteres gjennom den enkeltes skolehelsetjeneste på ordinær måte.
6. Tidligere engelske skjema foreligger nå i norsk oversettelse, dette gjelder deler av MSLQ skjemaet (management strategies, learning self-efficacy) og CCC-instrumentet (cross-curricular competencies).

Prosjektleders tilbakemelding er å anse som tilfredsstillende i forhold til komiteens merknader.

Vedtak
Komiteen godkjenner at prosjektet gjennomføres i samsvar med det som fremgår av søknaden.

Godkjenningen gjelder til 31.12.2017.

Av dokumentasjonshensyn skal opplysningene oppbevares i 5 år etter prosjektslutt. Forskningsfilen skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en datafil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato. Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren».

Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jf. helseforskningsloven § 10 tredje og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst A. Klagefristen er tre uker fra mottak av dette brevet, jf. forvaltningsloven § 29.

Med vennlig hilsen

Knut Engedal
Professor dr. med.
Leder

Anette Solli Karlsen
Komitesekretær

Kopi til: erik.kyrkjebo@hisf.no; post@hisf.no

APPENDIX II

ASK study information and consent form

06.03.2014, Sogndal

Kjære foreldre eller føresette i/ved 5. klassetrinn i Sogn og Fjorane, skuleåret 2014/15

Førespurnad om deltaking forskingsprosjektet «*ASK - Active Smarter Kids*»

KVA ER «ASK»?

ASK er eit stort utviklings- og forskingsprosjekt som skal undersøke korleis auka fysisk aktivitet i samspel med dei tradisjonelle faga påverkar skuleprestasjon, skuletrivsel og helse gjennom eitt skuleår (2014/15) for 5. klasseelevar.

Kva er formålet med ASK-prosjektet?

ASK-prosjektet er eit såkalla intervensjonsprosjekt som betyr at ein innfører noko nytt, for deretter å måle verknaden. For å måle verknad av ASK-modellen får halvparten av skulane intervensjonen (som er dagleg fysisk aktivitet) og den andre halvparten fortsetter som før. Skular der det er sju elevar eller meir på 5. klassetrinn i skuleåret 2014/15 vil bli inkludert i prosjektet. Skulane i kontrollgruppa 2014/15 vil få tilbod om same opplegg som prosjektgruppa, men eit år seinare (i 6. klasse, skuleåret 2015/16). Alle 26 kommunane i Sogn og Fjordane har sagt ja til deltaking i utviklings- og forskingsprosjektet ASK. Prosjektet vert gjennomført i samråd skuleregionane i Sogn og Fjordane og utdanningsaktørar i fylket. Kunnskapen som denne studien gjev vil vere viktig for å evaluere graden av kor fysisk aktive barn og unge bør vere med tanke på både læring og helse. ASK-prosjektet vil difor kunne gje samfunnet verdifull informasjon og kunnskap om organisering av skulekvardagen og metodar for førebyggande helsearbeid.

Kva inneber ASK-prosjektet for skulekvardagen til dykkar son/dotter dersom dykkar son/dotter går på ein skule som skal gjennomføre dagleg fysisk aktivitet?

Det faglege innhaldet i ASK-modellen (den daglege timen med fysisk aktivitet) blir utvikla i samarbeid mellom barneskulane i Sogn og Fjordane og HiSF, og inkluderer i løpet av ei skuleveke:

- 2 dagar x 45 minutt kroppsøving (dette gjeld alle elevar, både prosjektgruppe og kontrollgruppe)
- 1 dag x 45 minutt fysisk aktivitet (mest mogleg fysisk aktivitet på borna sine premiss)
- 3 dagar x 30 minutt «Aktiv læring» (elevane er fysisk aktive utandørs og øver på fag (t.d. mattebingo)
- 5 dagar x 5 minutt fysisk aktivitet i fag (elevane er aktive 5 minuttar i klasserommet kvar dag)
- 5 dagar x 10 minutt fysisk aktivitet i «aktiv heimelekse» (elevane er aktive 10 minutt kvar dag heime)

Den dagelge fysiske aktiviten er ikkje vurdert til å vere forbunden med risiko, og kan samanliknas med aktivitetar og metoder nytta i ein vanleg kroppsøvingstime.

Kva innber ASK-prosjektet for skulekvardagen til dykkar son/dotter dersom dykkar son/dotter ikkje går på ein skule som skal gjennomføre dagleg fysisk aktivitet?

For elevar ved skular som er kontrollgruppe vil skuleåret gå som normalt.

Kva inneber testing i ASK-prosjektet for dykkar son/dotter?

Det vil, ved oppstart (august/sepember 2014) og avsluttning (mai/juni 2015), bli gjennomført testar for å måle verknadar av ASK. Dette er derfor ein førespurnad til dykk som er foreldre eller føresette om ditt barn kan delta på ulike testar som målar verknadar av fysisk aktivitet på skuleprestasjon, skuletrivsel og helse i ASK-prosjektet.

Testane vert gjennomført i skuletida på dei lokale skulane eller på tilrettelagde testsenter i regi av HiSF. Tilhøva som blir undersøkt er alle knytt til skuleprestasjon, skuletrivsel og folkehelse. Dette inkluderer testar for kognisjon (testar som målar t.d. hukommelse og minne), ulike spørjeskjema, test av fysisk form og fysisk aktivitetsnivå, blodtrykk, motorikk, vekt og høgde. Det vil bli teke blodprøve. Foreldre/føresette blir spurde om å fylle ut eit spørjeskjema. Dersom ein elev sitt testresultatet visar avvikande medisinske verdiar vil skulehelsetenesta informerast og informasjonen til barn/foreldre vil ved desse tilfella komme frå skulehelsetenesta. Elevane i prosjektgruppa får fritak frå undervising slik at dei kan delta i testane. Dette er testar med låg eller ingen risiko for skader, og som er gjennomført og kvalititetsikra i fleire tilsvarande studiar. I tillegg til testane over, blir fire skular valt med på ei kvalitativ undersøking som inneber intervju og observasjon. Dersom dykkar son/dotter går i ein av desse skulane, vil han/ho få utdelt eit eige informasjonsskriv og samtykkjeerklæring for denne delen av prosjektet.

Frivillig deltaking i testar

Det er frivillig å ta del i testane i ASK-prosjektet. Ein kan trekkje seg frå heile eller delar av testane kva tid som helst og utan å oppgje grunn – utan at det får negative konsekvensar. De kan når som helst, og utan å oppgje nokon grunn, trekkje samtykke. Dette vil ikkje få konsekvensar for den vidare handsaminga av dykkar barn. Dersom foreldre/føresette eller dykkar son/dotter ynskjer å trekkje seg, vil innsamla data bli sletta.

Moglege føremoner og ulemper

Under alle testane blir det lagt vekt på barnet sitt beste, difor er testpersonellet som er ansvarleg for testane særs medvitne om at barn er ei sårbare gruppe. Alle moglege førehandsreglar blir tekne for å unngå eventuelle situasjonar som kan opplevast som ukomfortable for borna. Til dømes vil alle blodprøvar bli tekne i trygge lokale av røynde bioingeniørar. Me er medviten om at blodprøvetaking kan medføre psykisk påkjenningar for nokre av borna, og dersom barnet ditt ikkje ynskjer å ta blodprøven, men andre testar, er dette heilt i orden.

Kva skjer med informasjonen om dykkar barn?

Alle data som vert samla inn, både papirbasert og elektronisk, vert handsama i samsvar med krav til personvern og IKT-tryggleik nedfelt i helseforskingslova og personopplysningslova. Prøvane som ein tek og informasjonen som vert registrert om dykkar barn, skal berre nyttast i henhold til føremålet med studien. Alle skjema og data vert avidentifisert, det vil seie handsama utan namn og fødselsnummer eller andre direkte opplysningar som kan gjera at dei vert kopla til ditt barn. Identifiserbare opplysningar som knyter dykkar barn til opplysningane vert erstatta av ein kode. Lista som koplar kode og namn vert oppbevart på ein sikker måte åtskilt frå forskingsdataene, og berre prosjektleiinga har tilgang til namnelista og det er berre dei som kan finne attende til dykkar barn.

Kva skjer når prosjektet er avslutta?

Prosjektet vert avslutta 31.12.2016, men ASK ynskjer å oppbevare data for framtidige oppfylgingsstudium. Datamaterialet vil 31.12.2016 bli anonymisert for forskarar i ASK, men namnelista over prosjektdeltakarar og koden som koplar dei til data vert lagra hjå ein autorisert tiltrudd tredjepart, i dette høvet Personvernombodet for forsking hjå Norsk samfunnsvitenskapelig datatjeneste. Det eksisterer i dag ikkje tilfredsstillande kunnskap vedrørande langtidsverknadar av skulebaserte fysisk aktivitetsintervensjonar, og det kan derfor bli aktuelt at dykkar barn blir spurt om å delta ved eit seinare høve. Dersom dette blir aktuelt tek me kontakt.

Resultata av prosjektet vert publisert i form av engelskspråklege artiklar i internasjonal faglitteratur. I tillegg vil resultata frå prosjektet bli formidla til det norske fagmiljøet i form av populærvitskaplege artiklar og faglege føredrag. Me skal også skrive ein rapport frå prosjektet som er retta mot deltakarane og aktørar som har vore med på å legge til rette for gjennomføringa av prosjektet. Me understrekar at opplysningar som kjem fram i publikasjonar og føredrag ikkje kan førast tilbake til einskildpersonar.

Høgskulen i Sogn og Fjordane (HiSF) er ansvarleg for forskingsprosjektet, og vil gjennomføre all testing. Prosjektleiarar er førsteamanuensis Geir K. Resaland og professor Sigmund Alfred Anderssen. Prosjektet har vore gjennom ei grundig fagleg vurdering i Norges Forskingsråd som tildelte prosjektet 17,5 millionar kronar i oktober 2012 (prosjektnr. 221047). Norges Forskingsråd vurderte ASK-prosjektet til å ha svært høg kvalitet.

Dersom de aksepterer at dykkar barn tek del i testinga i ASK-prosjektet, skriv du under samtykkjeerklæringa på neste side. Om du seier ja til å vera med no, kan du seinare trekkje attende samtykkje utan at det påverkar handsaminga di elles. Dersom du seinare ynskjer å trekkje dykkar barn eller har spørsmål til studien, kan du kontakte Geir K. Resaland.

Dersom de på noko tidspunkt har spørsmål, ta gjerne kontant på telefon eller e-post.

Venleg helsing

Førsteamanuensis Geir K. Resaland
Tlf. 57676097, Mob. 41621333
e-post gk@hisf.no

Professor Sigmund Alfred Anderssen
Tlf. Mob. 45279348
e-post s.a.anderssen@nih.no

Samtykkje til deltaking i ASK-studiet

Eg har lese informasjonsskrivet og aksepterer at mitt barn tek del i ASK-studiet

---

(Signert av foreldre til prosjektdeltakar, dato)

Eleven sitt førenamn og etternamn: (Skriv tydeleg, helst med blokkbokstavar)

……..

Foreldre/føresette sitt førenamn og etternamn: (Skriv tydeleg, helst med blokkbokstavar)

……..

Eg stadfestar at eg har gjeve informasjon om studiet

Geir K. Resaland *, 6. mars 2014*

Signert, prosjektkoordinator Geir K. Resaland, dato

APPENDIX III

Supplementary tables and figures

Table A.1 Effects of the intervention on cardiometabolic risk factors (per-protocol analysis) (**paper III**)

	n	Group difference, β (95% CI)	*p*-value	ICC
WC	831	0.01 (-0.85 to 0.87)	0.98	0.19
SBP	833	-0.23 (-2.00 to 1.53)	0.79	0.09
TC:HDL-ratio	727	-0.02 (-0.11 to 0.08)	0.71	0.08
TG	727	0.03 (-0.03 to 0.10)	0.32	0.10
HOMA-score	718	0.14 (-0.14 to 0.42)	0.32	0.11
CRF	769	- 0.05 (-17.71 to 17.62)	0.97	0.14
Clustered risk score	624	-0.003 (-0.11 to 0.10)	0.95	0.14

Abbreviations; 95% CI: 95% confidence interval; β: beta-coefficient; CRF: cardiorespiratory fitness; HDL: high-density lipoprotein; HOMA: homeostatic model assessment; ICC: intraclass correlation coefficient (school); TC: total cholesterol; TG: triglyceride; SBP: systolic blood pressure; WC: waist circumference. Per-protocol: intervention schools reporting ≥ 80% of prescribed PA and control schools reporting < 120% of the curriculum prescribed PA.

Table A.2 Baseline physical activity levels and sedentary time by sex (**paper III**)

		Girls		**Boys**	
	n	Mean (±SD)	n	Mean (±SD)	*p*-value
PA during school	500		532		
PA total (cpm)		592 (162)		704 (187)	<0.001
MVPA (min/day)		25 (9)		33 (10)	<0.001
VPA (min/day)		10 (5)		13 (6)	<0.001
SED (min/day)		184 (18)		173 (19)	<0.001
PA all day	512		548		
PA total (cpm)		687 (239)		772 (31)	<0.001
MVPA (min/day)		68 (21)		81 (29)	<0.001
VPA (min/day)		27 (13)		34 (18)	<0.001
SED (min/day)		469 (57)		465 (62)	0.044

Abbreviations; MVPA, moderate-to-vigorous intensity PA; n, number; PA, physical activity, SED, sedentary time. Linear mixed models was used to analyse the between-gender differences in PA levels, adjusted for monitor wear time during school or all day, with school as a random effect to account for the cluster effect. Children having valid wear-time in ≥4 (out of 7) days and in ≥3 (out of 5) school days were included in the analysis.

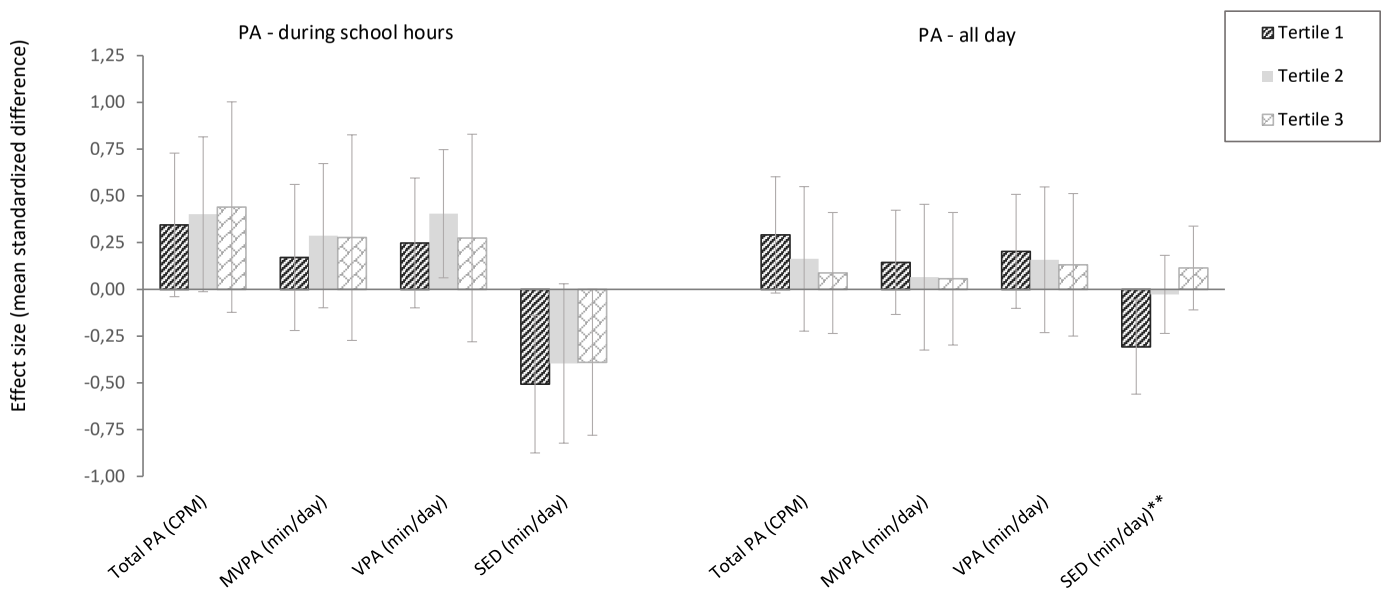

Fig. A.1 Subgroup differences in midway physical activity (PA) levels between the intervention and control group by tertiles (tertile 1 is the least favorable group and tertile 3 is the most favorable group in the cluster score at baseline) (**paper III**). Effect sizes are presented in standardized units (SD) ± 95% confidence intervals (CI). Linear mixed models was used to analyze the moderating effect by tertile, adjusted for monitor wear time during school or all day and baseline values of PA or sedentary behavior, with school as a random effect to account for the cluster effect. Children having valid data in all cardiometabolic risk variables and having valid wear-time in ≥4 (out of 7) days and in ≥3 (out of 5) school days were included in the analysis.
* Significant group x sex interaction ($p<0.1$). ** Significant group x sex interaction ($p<0.05$)

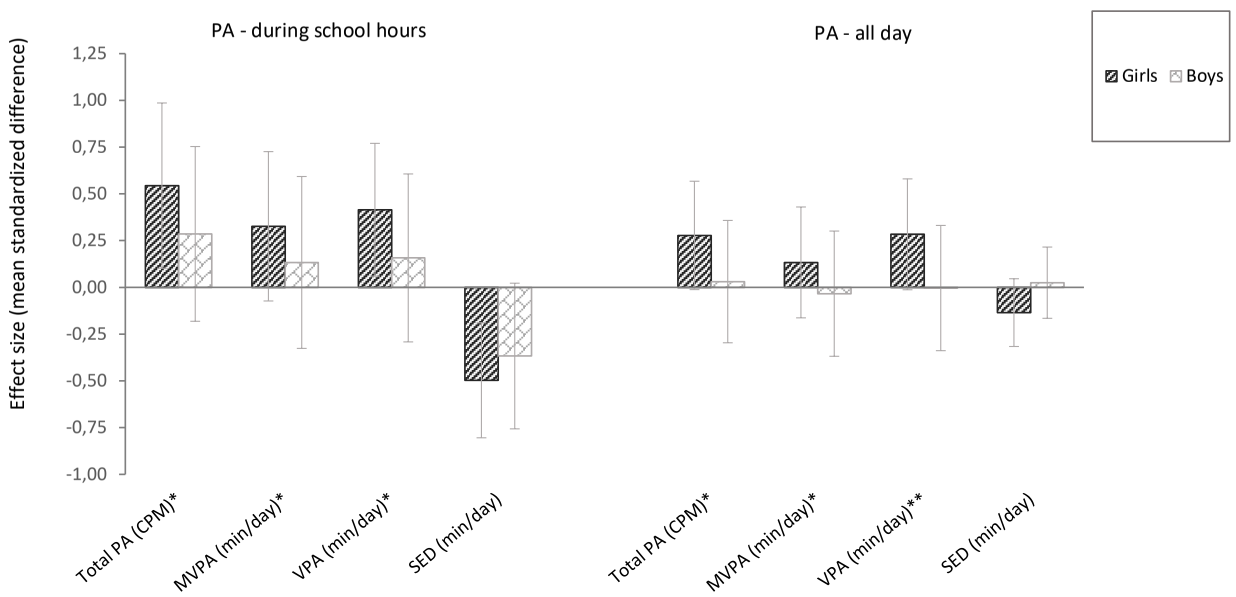

Fig. A.2 Subgroup differences in midway physical activity (PA) levels between the intervention and control group by sex (**paper III**). Effect sizes are presented in standardized units (SD) ± 95% confidence intervals (CI). Linear mixed models was used to analyze the moderating effect by sex, adjusted for monitor wear time during school hours or all day and baseline values of PA or sedentary behavior, with school as a random effect to account for the cluster effect. Children having valid data in all cardiometabolic risk variables and having valid wear-time in ≥4 (out of 7) days and in ≥3 (out of 5) school days were included in the analysis.
* Significant group x sex interaction ($p<0.1$). ** Significant group x sex interaction ($p<0.05$)